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## ORIGINAL ARTICLES

### INSECT ALLERGENS*: THEIR INTERRELATIONSHIP AND DIFFERENCES

#### A Comparison of Skin Reactivity and a Proposal for Routine Skin Testing With Insect Allergens

*Frank Perlman, M.D., Portland, Ore.*

With the broader acceptance of insects as inhalant allergens[1, 2] the problem has arisen of reducing the variety of testing material to workable and practical numbers for routine clinical studies. It must of course be solved without serious omissions or unnecessary duplications.

Arthropods are the most numerous group of animals in the world. The number of described species is approximately 80 per cent of all animals. Biologic classification is invariably complex. It is often controversial and sometimes arbitrary, but it is an attempt at orderliness. With insects it appears to be a never ending problem. Many changes have occurred through the years in the orders and families, but, at the level of genus and specie, changes and additions are continually occurring. This presentation proposes a practical path through the apparent entomological maze confronting most allergists. Conclusions and recommendations will be drawn from clinical observations correlated with basic entomological principles.

From the Allergy Clinic, 614 Portland Medical Center, Portland 5, Ore.
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*Common usage has caused the term "insect" to include most of the arthropods. This inexact terminology will be used throughout the discussion. It will conform with the broad title of the Insect Allergen Committee of the Research Committee of the American Academy of Allergy. When the class of true insects is singled out, the term "Hexapoda" will be used.

True insects (Class Hexapoda) are a class of the Phylum Arthropoda as indicated in Table I, and it is around this class that the major part of this discussion centers. According to Sabrosky,[3] at the United States National

TABLE I. PHYLUM ARTHROPODA[4]
(FIVE MAJOR CLASSES)

| | |
|---|---|
| Class Onychophora | (peripatus) |
| Class Myriapoda | (millipedes, centipedes, symphilides, etc.) |
| Class Crustacea | (crabs, shrimp, sowbugs, daphnia, etc.) |
| Class Arachnida | (spiders, mites, ticks, scorpions) |
| Class Insecta (Hexapoda) | (true insects) |
| Subclass Apterygota (wingless insects) | (silverfish, springtails) |
| Subclass Pterygota (winged insects) | (most common insects) |

Museum, the number of species throughout the world has been variously estimated at from 625,000 to 1,500,000. In North America the last figure was 82,000. These fall, according to Essig[4] of The Department of Entomology of The University of California, into 33 orders and are further divided into 839 families, as seen in Table II. Six of these 33 orders embrace 96 per cent of the known species which surround us and whose distribution is world-wide. It therefore becomes evident that the list of insects to arouse clinical concern may be quickly narrowed.

TABLE II. ORDERS OF TRUE INSECTS[3, 4]
(SIX OF THESE EMBRACE 96 PER CENT OF DESCRIBED SPECIES)

| ORDER | COMMON NAMES | FAMILIES (APPROX. NO.) | PER CENT OF SPECIES | NUMBER OF SPECIES | |
|---|---|---|---|---|---|
| | | | | WORLD-WIDE | NORTH AMERICA |
| Coleoptera | beetles, weevils | 154 | 40 | 277,000 | 26,076 |
| Lepidoptera | moths, butterflies | 138 | 17 | 112,000 | 16,300 |
| Hymenoptera | bees, wasps, ants | 151 | 15 | 103,000 | 14,528 |
| Diptera | flies, mosquitoes | 144 | 13 | 86,100 | 16,938 |
| Hemiptera, Heteroptera, Homoptera | true bugs, aphids, scales, cicadas | 72 | 8 | 55,000 | 8,742 |
| Orthoptera | grasshoppers, cockroaches, etc. | 21 | 3 | 23,600 | 1,033 |
| Minor orders | | 159 | 4 | 28,200 | 3,865 |
| | | 839 | 100 | 684,900 | 87,482 |

These insects vary in numbers on a geographical and seasonal basis. It is not difficult to gain knowledge of the appearance of certain insects in large numbers in an area at a specific period. Regional entomologists can often anticipate such occurrences. If clinical symptoms were to occur at such times (as we witness with pollens or even with such insects as caddis fly and May fly[5, 6, 7]), then current entomological information would be most valuable. There is as yet no well-documented evidence of the occurrence of hay fever and asthma in relation to the appearance of large numbers of insects other than the caddis fly and the May fly. However, in this discussion it is presumed

that those remaining insects not immediately implicated may become, over a period of time, inhalant allergens in the house and outdoor dust as part of the debris contaminating the air we breathe. Without a reliable technique for detecting such insect debris by air sampling (as can be done for pollens) it becomes necessary to accept the ubiquitous nature of certain insects together with positive skin tests and the clinical picture to correlate insect allergens to symptoms. Such presumptions have led to the establishment of a routine in our clinical studies.

The search for common excitants to embrace a larger group of potential allergens must begin at some level; it may broaden or narrow on the basis of clinical experiences. The use of biologic classification for establishing common allergenicity among insects may be fortuitous. The laborious task to find common allergenicity through such immunologic methods as gel diffusion studies would still fail to provide acceptable clinical inferences. The specific skin reaction is a more sound basis for presuming their potential or actual clinical importance as inhalant allergens. The additional search for relationship through neutralization or exhaustion studies with related insects add interest but interpretations of such studies have their shortcomings.[8]

## METHODS

Our studies and conclusions were concerned with insects as inhalant allergens. We presumed that their allergenic quality could be detected by positive skin reactions and the presence of skin sensitizing antibodies in the patient. The phenomena associated with the bite or sting of certain insects are not included in this presentation since there are no consistently demonstrable antibodies of the reagin type in that form of insect allergy. The various insect material was used as it became available. Uniformity was sought by making all extracts in the same method from the live or frozen state. The final dried, powdered material after lyophilization and defatting was stored under desiccation until used. Aqueous extracts for testing were made from the stabile powder using 50 per cent glycerinated buffered solution in a 1:10 dilution by weight volume. After passing through a sterilizing Seitz filter and with storage under ordinary refrigeration these extracts were presumed to remain stabile and actively allergenic throughout our studies.[9, 10] There was no significant nonspecific irritative reaction to any of these extracts.

Most of the various insects used in these studies are listed in Table III. Division was reduced to the level of family. To avoid making the tables cumbersome, only a few of the families have been listed. For example, in the order Coleoptera (beetles) and in the order Lepidoptera (moths) more than a dozen families were tried in each and showed similar but varying degrees of skin reactions. Sometimes a number of representatives of such a family available in small amounts were combined in order to supply an adequate quantity of material for extracting. In most instances a single representative of the family was used and is so indicated.

TABLE III. INSECT MATERIAL USED IN THESE STUDIES

| ORDERS | FAMILIES | COMMON NAMES |
|---|---|---|
| Class Hexapoda (Insecta) | | |
| 1. Ephemeroptera | — | May flies |
| 2. Orthoptera | Acrididae | grasshoppers |
| | Blattidae | cockroaches |
| 3. Isoptera | — | termites |
| 4. Dermaptera | — | earwigs |
| 5. Hemiptera | Coreidae | box-elder bugs, also 6 minor families |
| 6. Homoptera | Cicadellidae | leafhoppers, aphids |
| | Aphididae | |
| 7. Coleoptera | Dermestidae | carpet beetles, June beetles, ground beetles, also 12 minor families |
| | Scarabeidae | |
| | Carabidae | |
| 8. Neuroptera | Sialidae | lacewings |
| 9. Trichoptera | — | caddis flies |
| 10. Lepidoptera | Tineidae | clothes moths |
| | Noctuidae | noctuid moths |
| | Bombycidae | silkworm moths, also 10 minor families |
| 11. Diptera | Muscidae | houseflies |
| | Culicidae | mosquitoes |
| | Cecidomyiidae | midges |
| | Tabanidae | deer flies |
| | Simuliidae | black flies, also 4 minor families |
| 12. Siphonaptera | Dolichopsyllidae | fleas (rat) |
| 13. Hymenoptera | Apidae | honeybees |
| | Bombidae | bumblebees |
| | Vespidae | wasps, hornets, yellow jackets |
| | Formicidae | ants (black) |
| Class Arachnida | | |
| 1. Scorpionida | | scorpions |
| 2. Aranea | | spiders |
| 3. Acarina | | tick of Rocky Mt. spotted fever |
| Class Crustacea | | |
| 1. Decapoda | | lobster, crab, shrimp |
| 2. Copepoda | | plankton shrimp |
| 3. Branchiopoda | | daphnia, fairy shrimp |
| 4. Isopoda | | sowbugs |

Over a period of 2 years, 728 patients were tested with various insect extracts that were on hand. The expanding list of materials slowly gathered made statistical analysis of very limited value. It soon became apparent, however, that a recurrent pattern of relationship and differences in skin reactivity to the insects existed. This was pursued further by re-testing patients chosen because of previous strong skin reactions as well as because of the presence in their sera of skin sensitizing antibodies. Approximately the same degree of whealing reaction was repeatedly elicited among families within an order.

OBSERVATIONS

The following most consistent and significant observations are offered. Random cases with skin reactions exemplify these observations in the accompanying tables.

1. Multiple reactions occurred in a number of patients to most of the insect extracts used. Where such multiple reactions occurred, the least reactive insects were in the isolated orders or in orders not abundantly present in the patient's environment—Isoptera (termites), Dermaptera (earwigs), Ephemeroptera (May fly), Trichoptera (caddis fly).

2. Among patients with multiple reactions most of these occurred within a single class. This sometimes fell within the class Hexapoda and at other times within the class Arachnida, or the class Crustacea (Table IV).

TABLE IV. REACTIONS TO SEVERAL ORDERS WITHIN A CLASS

| PATIENT | CLASS | ORDER | COMMON NAME | REACTION |
|---|---|---|---|---|
| W. B. | Arachnida | Aranea | spider | ++++ |
| | | | spider web | ++++ |
| | | Scorpionida | scorpion | ++++ |
| | Hexapoda | Lepidoptera | moth | − |
| | | Diptera | mosquito | − |
| | | Hymenoptera | honeybee | − |
| R. K. | Crustacea | Isopoda | sowbug | ++++ |
| | | Branchiopoda | daphnia | ++++ |
| | | Decapoda | crab | ++++ |
| | | Copepoda | shrimp (plankton) | +++ |
| | | | shrimp eggs | − |

3. Periodically isolated or single reactions within a class or even within a single order occurred (Table V).

TABLE V. PERIODIC ISOLATED REACTION OF A SINGLE ORDER WITHIN A CLASS

| PATIENT | CLASS | ORDER | COMMON NAME | REACTION |
|---|---|---|---|---|
| A. S. | Hexapoda | Orthoptera | grasshopper | ± |
| | | Homoptera | leafhopper | ± |
| | | Coleoptera | June beetle | − |
| | | Lepidoptera | moth | ± |
| | | Diptera | mosquito | − |
| | | Hymenoptera | honeybee | ++++ |
| C. P. | Hexapoda | Orthoptera | grasshopper | ± |
| | | Isoptera | termite | − |
| | | Dermoptera | earwig | +++ |
| | | Coleoptera | June beetle | − |
| | | Diptera | housefly | − |
| P. B. | Hexapoda | Isoptera | termite | +++ |
| | | Hymenoptera | ant | ± |
| | | Lepidoptera | moth | ± |

4. Those patients reacting to a single order of insects within a class reacted to some degree to all of the families represented. Variation in degree of reaction to various families may have been in part the result of variation in allergenicity of the extract used, or possibly the presence of other allergens unrelated to the insect itself, as might be the case with the honeybee (Table VI).

TABLE VI. REACTIONS OF REPRESENTATIVE FAMILIES WITHIN EACH ORDER

| PATIENT | CLASS | FAMILY | COMMON NAME | REACTION |
|---|---|---|---|---|
| G. D. M. | Hymenoptera | Apidae | honeybee | ++++ |
| | | Vespidae | hornet | +++ |
| | | Vespidae | yellow jacket | ++++ |
| | Lepidoptera | Noctuidae | moth | – |
| T. L. | Homoptera | Cicadelladae | leafhopper | +++ |
| | | Aphididae | aphid | +++ |
| | Dermoptera | | earwig | – |
| | Coleoptera | | June beetle | – |
| | Trichoptera | | caddis fly | – |
| L. E. | Homoptera | Aphididae | aphid | ++++ |
| | | Cicadelladae | leafhopper | ++++ |
| | Orthoptera | Blattidae | cockroach | ± |
| | | Acrididae | grasshopper | ± |
| | Diptera | Muscidae | housefly | +++ |
| | | Culicidae | mosquito | ++++ |
| | | Cecidomyiidae | midge | ++++ |
| | | Tabanidae | deer fly | +++ |
| G. L. | Diptera | Muscidae | housefly | ++ |
| | | Culicidae | mosquito | +++ |
| | | Tabanidae | deer fly | +++ |
| | | Simuliidae | black fly | ++ |
| | Lepidoptera | | moth | ± |
| | Ephemeroptera | | May fly | – |
| | Trichoptera | | caddis fly | – |
| | Homoptera | | leafhopper | – |
| P. R. | Orthoptera | Acrididae | grasshopper | ++++ |
| | | Blattidae | cockroach | ++++ |
| | Lepidoptera | | moth | ++ |
| | Diptera | | mosquito | ++ |
| | Dermoptera | | earwig | – |
| | Hymenoperta | | wasp | – |

5. Reaction to one or two families and with less or no reactions to others within the same order occurred only in a few cases (Table VII).

6. Comparable anatomic structures and secretory products (silk, venom, etc.) showed no consistent allergenic interrelationship between classes.

TABLE VII. OCCASIONAL REACTIONS TO ONE OR TWO FAMILIES AND NOT TO ALL WITHIN AN ORDER

| PATIENT | ORDER | FAMILY | COMMON NAME | REACTION |
|---|---|---|---|---|
| D. K. | Diptera | Muscidae | housefly | ++++ |
| | | Culicidae | mosquito | ± |
| | | Cecidomyiidae | midge | ++ |
| | | Tabanidae | deer fly | +++ |
| A. P. | Diptera | Muscidae | housefly | +++ |
| | | Culicidae | mosquito | +++ |
| | | Cecidomyiidae | midge | – |
| | | Tabanidae | deer fly | + |
| W. W. | Hymenoptera | Apidae | honeybee (worker) | ++++ |
| | | Vespidae | hornet | – |
| | | | yellow jacket | ± |
| | | Formicidae | ant | – |
| | | Apidae | honeybee (drone) | ++++ |
| | | Bombidae | bumblebee | +++ |

7. Unusual observation continually occurred as exemplified by (a) reaction to silk and not to moth and vice versa,[2] (b) reaction to shrimp egg and not to the hatched plankton (brine) shrimp from the same lot of eggs, and vice versa, and (c) reaction to spider and not to spider web (Table VIII).

TABLE VIII. REACTIONS TO ANATOMIC STRUCTURES AND SECRETORY PRODUCTS

| PATIENT | CLASS | ORDER | COMMON NAME | REACTION |
|---|---|---|---|---|
| W. B. | Hexapoda | Lepidoptera | silkworm silk | – |
| | Arachnida | Aranea | spider web | ++++ |
| | Hexapoda | Lepidoptera | caterpillar web | ± |
| D. T. | Arachnida | Aranea | spider | ++++ |
| | | | spider web | +++ |
| | | Scorpionida | scorpion | ++ |
| | Hexapoda | Diptera | housefly | – |
| | | Lepidoptera | moth | – |
| | | Coleoptera | beetle | + |
| D. K. | Arachnida | Aranea | spider | ++++ |
| | | | spider web | ± |
| | | Scorpionida | scorpion | ++++ |
| | Hexapoda | Lepidoptera | moth | +++ |
| | | | caterpillar | +++ |
| | | | caterpillar web | – |
| M. B. | Hexapoda | Lepidoptera | silk | ++++ |
| | | | silk moth | – |
| G. R. | Crustacea | Isopoda | sowbug | – |
| | | Branchiopoda | daphnia | – |
| | | Copepoda | shrimp (plankton) | – |
| | | | shrimp eggs | +++ |

8. Exhaustion tests with serum from several extremely sensitive patients suggested differences in allergenicity between orders. These demonstrations of exhaustion were fraught with contradiction.[8] Some observations with exhaustion tests, however, such as that shown in Table IX, suggest that each order of insects may possess reagin-stimulating allergens both common to and differing from those of other orders.

TABLE IX. EXHAUSTION TEST
(SERUM OF P. R.* TO H. W.* SITE PREPARED DEC. 7, 1957)

| SITE | PREPARED WITH | DAYS AFTER INITIAL SITE PREPARATION | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2 | 3 | 4 | 5 | 6 | 7 |
| A | P. R. serum | 12-9-57 | 12-10-57 | 12-11-57 | 12-12-57 | 12-13-57 | 12-14-57 |
| | | G. H. | G. H. | G. H. | No testing | | C. R. |
| | | ++++ | ++ | ± | | | – |
| B | P. R. serum | G. H. | G. H. | G. H. | A and B | | M. |
| | | ++++ | ++ | ± | still indurated | | ++ |
| C | P. R. serum | | | | | | C. R. |
| | | | | | | | +++ |
| D | P. R. serum | | | | | | M. |
| | | | | | | | +++ |
| E | Unprepared | | | | | | C. R. |
| | | | | | | | – |
| F | Unprepared | | | | | | M. |
| | | | | | | | ± |

Antigen used: G. H. (grasshopper); M. (moth); C. R. (cockroach).
Serum of P. R.
*P. R., patient; H. W., recipient.

DISCUSSION

A comparison of interrelated antigenicity among the insects to that among plants or vertebrates offers interesting though vulnerable analogies. Among hayfever producing plants the families Amaranthaceae and Chenopodaceae have pollens which are structurally and antigenically closely related (Russian thistle and pigweed). The families within the large heterogeneous group of composite plants all frequently react to some degree on sensitive individuals, and yet among grasses (the family Gramineae) the skin reactions may show wide variation on a clinically sensitive patient with one tribe of grasses producing much larger whealing reaction than another. This is probably more a matter of degree rather than differences in allergens.

In the animal kingdom within the class Mammalia the dander of cow, deer, and camel, all within the suborder Ruminantia, usually react on patients sensitive to cow hair. Within the order Carnivora and its suborder Tissipedia, the cat and dog families both often react to varying degrees in a patient sensitive to one or to the other.

TABLE X. PROPOSED LIST OF INSECTS FOR ROUTINE TESTING

| ORDER* | COMMON NAMES OF INSECTS† |
|---|---|
| Class Hexapoda | |
| 1. Coleoptera | beetles, weevils |
| 2. Lepidoptera | moths, butterflies |
| 3. Hymenoptera | honeybees, hornets, yellow jackets |
| 4. Diptera | flies, mosquitoes |
| 5. Orthoptera | grasshoppers, locusts, cockroaches |
| 6. Hemoptera | box-elder bugs, squash bugs |
| 7. Homoptera | aphids, leafhoppers, cicadas, scales |
| 8. Minor orders | mixture of common representative |
| Class Arachnida | |
| 9. Aranea | spiders |
| 10. Acarina | mites |
| Class Crustacea | |
| 11. Isopoda | sowbugs |
| 12. Branchiopoda | daphnia, fairy shrimp |

*The first four orders will encompass 80 per cent of the common species found in North America.

†Families and species not indicated. A mixture of those most abundant and most readily available may be used.

TABLE XI. REGIONAL AND LESS COMMON INSECTS FOR SPECIFIC PROBLEMS

| | |
|---|---|
| Class Hexapoda | |
| 1. Order Ephemeroptera | May flies |
| 2. Order Trichoptera | caddis flies |
| 3. Order Neuroptera | lacewings |
| 4. Order Thysanura | silverfish |
| Class Myriapoda | |
| 5. Order Chilopoda | centipedes |
| 6. Order Diplopoda | millipedes |
| Class Crustacea | |
| 7. Order Copepoda | plankton (brine) shrimp |

Classification of plant and animal life is based on many factors including anatomy, physiology, biology, ecology, and even cytology. The allergist who must accept allergenic relationship only on the basis of immunologic similarity must, however, be impressed by a close relationship between the taxonomist's arrangement and his own observation, particularly with skin testing. There is thus a strong temptation to use entomological classification in grouping our skin testing with insects.

The arbitrary but apparently valid acceptance of the classification level of order for grouping of tests, together with the reduction of the orders to those embracing 96 per cent of the known species, has brought the list of important insects to a less formidable number. It has reduced the number of recommended tests to a mere dozen (Tables X and XI). This should make the task of routine studies with insect allergens easier.

## SUMMARY AND CONCLUSIONS

1. Allergenic interrelationship of insects at the level of orders and families is suggested as a result of extensive skin testing.

2. Routine "screening" for probable sensitivity to insects would require about 12 of the most common classes and orders of Arthropods.

3. A proposed list of extracts for routine testing is offered.

4. More detailed testing with various families, genuses or even species within a reacting order may follow and may reveal occasional variation in allergenicity.

The author wishes to acknowledge the invaluable assistance in obtaining insect material and guidance through the entomological maze by Dr. Charles E. Woodworth, Walla Walla, Washington, Consulting Entomologist to the Insect Allergen Committee of the Research Council Committee of the American Academy of Allergy.

## REFERENCES

1. Feinberg, A. R., Feinberg, S. M., and Benaim-Pinto, C.: Asthma and Rhinitis From Insect Allergens, J. ALLERGY **27**: 437, 1956.
2. Perlman, F.: Insects as Inhalant Allergens, J. ALLERGY **29**: 302, 1958.
3. Sabrosky, C. W. (United States Dept. of Agriculture): Insects, Yearbook of Agriculture, Washington, 1952, U. S. Government Printing Office.
4. Essig, E. O.: College Entomology, New York, 1942, The Macmillan Company.
5. Figley, K. D.: Asthma Due to May Fly, Am. J. M. Sc. **178**: 338, 1929.
6. Osgood, H.: Allergy to Caddis Fly (Trichoptera). I. The Insect, J. ALLERGY **28**: 113, 1957.
7. Osgood, H.: Allergy to Caddis Fly (Trichoptera). II. Clinical Aspects, J. ALLERGY **28**: 292, 1957.
8. Pruzansky, J. J., Feinberg, S. M., Haminker, A., and Meleyco, L. N.: Antigenic Relationship of Insect Extracts, J. ALLERGY **30**: 352, 1959.
9. Hampton, S. F., Johnson, M. C., Frankel, S., and Schiele, A. W.: Preparation of Highly Concentrated Allergenic Extracts by Increasing the Concentration of Extraction. I. Timothy and Low Ragweed Pollens, J. ALLERGY **26**: 16, 1955.
10. Perlman, F.: Allergenic Extracts—A Comparison of Their Quality and Reliability, J. ALLERGY **30**: 24, 1959.

# SKIN RESPONSE TO HISTAMINE OF THE PROTEIN-SENSITIZED GUINEA PIG

*R. St. J. Buxton, M.B., Ph.D., Bristol, England*

At a symposium in 1956, Perry[15] compared the skin reactivity of different species and noted that a high degree of response was not associated with a high histamine level. In an earlier report, however, Brocklehurst, Humphrey, and Perry[1] stated that a considerably reduced response to histamine was obtained in rats after administration of Compound 48/80, and this suggested that the species effect was the dominant feature. Guinea pigs were shown by Feldberg and Miles[4] to have a low skin content of histamine, whereas their degree of skin reactivity in allergic manifestations is acknowledged to be high. Brocklehurst and his associates[1] reported unpublished work by Perry which showed that the skin histamine of guinea pigs was resistant to histamine liberators. In the article by Feldberg and Miles,[4] mention is made of Miles' experiments demonstrating a rise in skin histamine after sensitization. In view of this, it was decided to carry out studies on the response to histamine of the hypersensitive guinea pig.

## METHODS

Histamine sensitivity of the guinea pig skin was examined by the method of Miles and Miles[9] in which pontamine sky blue was injected intravenously, histamine being subsequently given intradermally into the depilated skin. Pontamine sky blue was made up as a 5 per cent solution and each guinea pig received 70 mg. per kilogram of body weight two hours before testing. The animals' backs were prepared and 0.1 ml. of histamine acid phosphate containing 1 $\mu$g, and 0.1 ml. of physiological saline as the control substance, were each injected into eight randomly determined sites. After thirty minutes the diameter of each blue area was measured and the total for histamine and for the control was obtained. The ratio of histamine to control responses was then calculated. The effect of a treatment was studied using batches of at least six nonpregnant female guinea pigs, weighing 500 to 700 grams, and the results were assessed by applying the analysis of variance.

From the Department of Physiology, University of Bristol, Bristol 8, Engand.

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*Sensitization.*—Horse serum, 0.5 ml., was given intraperitoneally and the animals were examined twenty-one to twenty-four days later. The sensitivity was tested after the experiment, using terminal ileum in the isolated organ bath and, in the absence of a positive response, the results for that animal were discarded.

*Tissue Analysis.*—The guinea pig tissues were loaded with sodium by injection intraperitoneally of 0.2 ml. per 100 grams of body weight of a saturated sodium chloride solution. The experimental procedure was undertaken half an hour later.

*Water and Electrolyte Depletion.*—The method of Yannet and Darrow[20] was used to deplete the guinea pig tissues of water and electrolytes. Intraperitoneal injections of 10 ml. per 100 grams of body weight of a 5 per cent glucose solution were made and the studies were made three hours afterward.

*Drug Administration.*—Hydrocortisone,* 5 mg. in aqueous suspension, was injected intramuscularly twenty hours before the tests were made.

Ascorbic acid,† 2.5 mg. in aqueous solution, was given intramuscularly two hours before the examination.

Ro 3-0809,† a phenyl benzyl phosphate derivative with an anti-hyaluronidase action, was injected subcutaneously in doses of 2.5 mg. per 100 grams, of body weight as a 1 per cent solution half an hour before testing.

AHT,† which has the form of 8-chloro-2 methyl-1:3:4:5-tetrahydro-2 pyrid-[4, 3, b] indole hydrochloride, was given subcutaneously half an hour before the experiment in a 1 per cent aqueous solution using 2.5 mg. per 100 grams of body weight. This substance has a peripheral serotonin antagonistic effect and, in this dosage, appeared to produce no other relevant changes.

Assuming that 8 per cent of body weight of the guinea pig was blood, 0.01 mg. per milliliter of blood of d-lysergic acid diethylamide tartrate‡ was injected subcutaneously. The tests were performed half an hour later.

## RESULTS

*Effect of Sensitization.*—The blue areas on the backs of the normal guinea pigs were about three times larger in response to the histamine than to the control solution. This is shown in the right column of Fig. 1. When the observations on eight normal and eight sensitized animals were compared, the histamine response was found to have increased in the latter (Fig. 1) by four times without an appreciable change in the control value. The ratio of histamine to control diameters was therefore raised to a significant extent ($P \cong 0.02$) by sensitization.

*Effect of Electrolyte Change.*—An earlier paper (Buxton and McDowall[2]) reported that, after protein sensitization, the skin of the guinea pig had a

*From Roussel Laboratories, Harrow Road, London.
†From Roche Products Ltd., Welwyn Garden City, Herts.
‡From Sandoz Products, Great Castle St., London.

lower sodium content than that of the normal animal. But when the normal animal was subjected to water and electrolyte depletion by means of hypertonic glucose solution given intraperitoneally, the response to histamine was little changed, and the increase in the histamine to control ratio did not reach a significant level. In addition to this, the procedure used to raise the skin sodium content was accompanied by a greater spread of dye after injections of both histamine and saline, with a wide range of response. The ratio of the histamine to saline diameters was not altered to a significant extent.

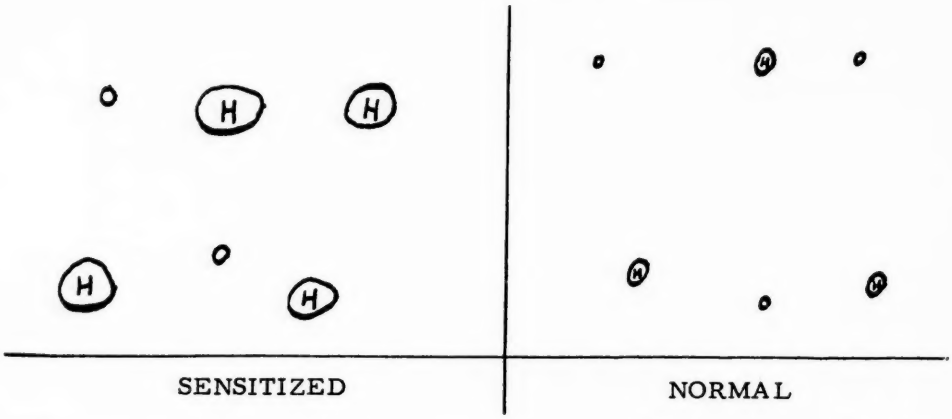


Fig. 1.—Part of the injected fields of a sensitized and normal guinea pig for comparison of the histamine response *(H)*. The control injections are unmarked.

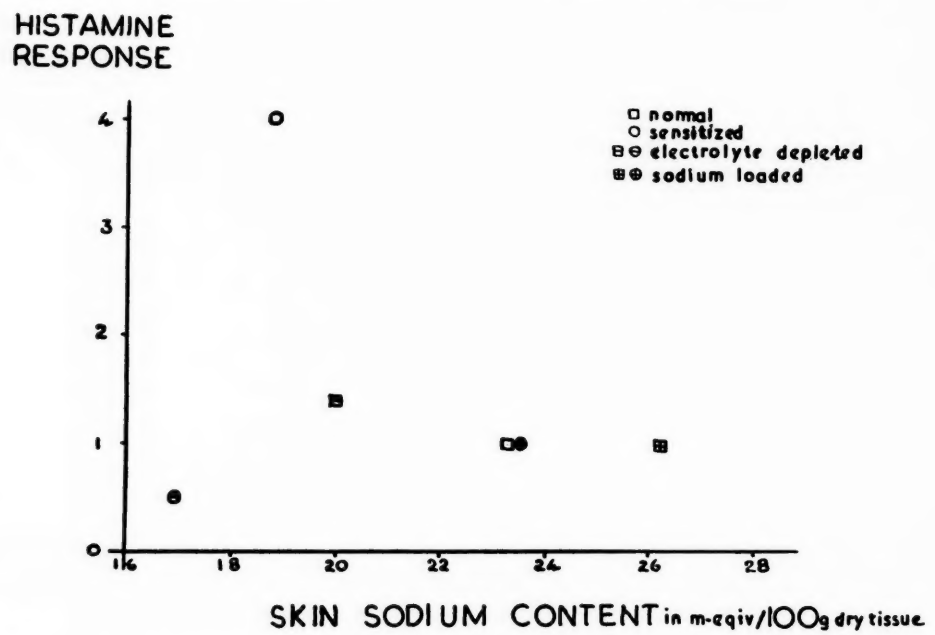


Fig. 2.—The relationship between the histamine response using an arbitrary scale and the guinea pig skin sodium content.

Conversely, the sensitized animals receiving a sodium load showed a reduction in histamine response without appreciably changing the control areas. In this way, the ratio of the responses fell to a quarter of the former value, which was a significant difference ($P < 0.01$). If, however, the electrolyte content of the skin was lowered by a hypertonic glucose injection, the dye spread was increased after injections of histamine and saline, but, as the latter effect was the greater, the ratio of histamine to saline areas was reduced to a significant extent ($P < 0.01$).

Fig. 2 shows these results in graphic form. The histamine-saline ratio of the normal animal is assumed to be unity and the experimental observations are presented with reference to this. The skin sodium content is given in milliequivalents per 100 grams of dry tissue. No obvious relationship between the sodium level in the skin and the histamine response is apparent. In the normal animals the effect of histamine was little changed by the experimental procedures but the reaction in sensitized guinea pigs was reduced.

*Effect of Drugs.—*

*Anti-hyaluronidase:* Changes in tissue permeability can account for an increased spread of a dye in a tissue. Duran-Reynals' work on Spreading Factor, which led to the identification of hyaluronidase, described such a factor in skin (Claude and Duran-Reynals[3]). Studies were therefore undertaken using a drug to inhibit hyaluronidase and so obtain information as to whether the increase of dye spread in sensitized guinea pigs in response to histamine could be attributed to this enzyme.

After injections of Ro 3-0809 in twenty-four guinea pigs, the twelve sensitized animals showed a reduced response to histamine, the histamine-control ratio falling to the level of the untreated normal animals, which represents a highly significant effect ($P < 0.01$). In the twelve normal guinea pigs no significant change was found after this treatment.

*Hydrocortisone:* Kellaway and Cowell[8] first reported the enhanced sensitivity to histamine of the adrenalectomized cat. It seemed logical therefore to determine whether the increased response of the sensitized guinea pig was reduced by the appropriate adrenal hormone. Hydrocortisone did, in fact, diminish the histamine-saline ratio by a significant extent ($P < 0.02$), but mainly by increasing the effect of the control solution. The ratio was not, however, brought to the normal level. The normal guinea pigs treated with hydrocortisone presented small changes after histamine and saline injections so that the ratio was increased but not to a significant extent.

*Ascorbic acid:* The relationship between ascorbic acid and the adrenocortical hormones is still a matter for debate. Perla found that histamine resistance was not increased in the adrenalectomized rat by giving ascorbic acid.[12] But he found that a scorbutic diet increased the sensitivity and a single massive injection of ascorbic acid conferred some protection against protein shock (Perla and Marmorston[13]). The results in our experiments of a single administration of ascorbic acid to normal and sensitized guinea pigs

were similar to those obtained by giving hydrocortisone, namely a reduced response in the sensitized and an increased one in the normal group; but, since the observations were less scattered, these were significant at the 1 and 5 per cent levels, respectively.

*Antiserotonin:* The anaphylactoid reaction in rats was shown by Parratt and West[11] to be due to the release of 5-hydroxytryptamine. As a state of hypersensitivity precedes anaphylaxis, the effect of an antagonist to serotonin on the sensitized animal was considered worthy of examination. AHT reduced the histamine response dramatically in both normal and sensitized guinea pigs, the ratio of histamine to saline being significantly lowered ($P < 0.01$).

This finding was further examined in the sensitized animals by giving lysergic acid diethylamide which antagonizes 5-hydroxytryptamine. The results confirmed the observations with AHT, the histamine response being decreased by a significant amount ($P < 0.01$).

The ratios of histamine to saline measurement under the different treatments are summarized in Table I. The value for the normal animals was taken as unity as in compiling Fig. 2, and the remaining observations were calculated in relation to this.

TABLE I. HISTAMINE: SALINE RATIOS OF BLUED AREAS WITH DIFFERENT TREATMENTS

| | UNTREATED | SODIUM-LOADED | SODIUM-DEPLETED | HYDROCORTISONE | ASCORBIC ACID | ANTI-HYALURONIDASE (RO 3-0809) | ANTI-SEROTONIN (AHT) | LSD |
|---|---|---|---|---|---|---|---|---|
| Normal | 1.0 | 2.5 | 1.4 | 2.2 | 1.5* | 0.8 | 0.5* | — |
| Sensitized | 4.0* | 1.0* | 0.5* | 1.8* | 2.2* | 2.7* | 0.3* | 2.2* |

*These findings represent a significant change.

## DISCUSSION

Perry[14] found that the skin histamine of the normal guinea pig was resistant to histamine liberators. The effect of protein sensitization was to raise the skin histamine content (Miles[10]), but whether the increase in histamine response, which is reported above, was due to this or to other changes provoked by the sensitization process is not yet clear. The effects might either take the form of an exaggerated response or of a response which differed qualitatively. Since Brocklehurst and his co-workers,[1] using rats given Compound 48/80 which liberated 90 per cent of the total skin histamine which is all that is able to be released, were still able to elicit the Arthus phenomenon and passive cutaneous anaphylaxis, a substance other than histamine must have been involved. Fink[5] demonstrated the complete inhibition of the anaphylactic contraction of the uterus of the sensitized mouse by LSD and reserpine, and Fischer and Lecomte[6] found that after anaphylactic shock in rabbits the urinary excretion of 5-HIAA was raised. These experiments among other reports suggest that 5-hydroxytryptamine was involved in anaphylactic responses in the rat, the mouse, and the rabbit. The studies reported, although on the hypersensitive guinea pig, showed an effect on the histamine response

after both the 5H-T antagonists. These results would suggest that not only is 5H-T concerned in the increased response to histamine after sensitization but that it also plays a part in the response in normal guinea pigs. The skin in normal guinea pigs has few mast cells over the area studied and there is at present no evidence that serotonin is found in these cells in the guinea pig. Riley and West,[17] in fact, showed that intravenous administration of histamine in rats did not produce mast cell damage in normal dosage. It would seem, therefore, that mast cells are not involved in this type of response in the rat. If the same is true in the guinea pig, there is no reason to comment on the relationship of serotonin and the mast cell, or the absence of mast cells in the skin, when considering the histamine response in the guinea pig skin. The location of serotonin in the skin and the mechanism of its release after histamine injection remain obscure.

The change in histamine response which was brought about by hydrocortisone was not a significant one in the normal guinea pigs. This finding is in agreement with that of Miles and Miles[9] who used cortisone. In the sensitized animals, however, a significant reduction in effect was demonstrated. Sudak, Wyman, and Fulton[19] found that cortisone gave some protection against large doses of histamine and it may be that an increased sensitivity to histamine in the sensitized animals evoked a similar type of response.

The state of sensitization in the guinea pig was shown to be associated with alteration in some tissue constituents (Buxton and McDowall[2]). This paper reported only on the water, sodium, and potassium content of certain tissues, including the skin. Procedures expected to change these constituents did not effect the histamine response in such a way as to lend suport to the suggestion that the electrolyte content of the skin and the histamine response were related, but it may be that other tissue changes are more imporant in this respect. A reduction in the hyaluronic acid or an increase in the hyaluronidase, for example, would be an interesting finding in view of the fact that the hyaluronidase antagonist was effective in reducing the histamine response in the sensitized guinea pigs but not in the normal animals. Certain of our other observations would fit in with such a theory. Seifter and associates[18] observed that cortisone opposed the action of hyaluronidase when the permeability of synovial membrane was used to examine this, and Hayes and Baker[7] reported the reduction of dye spread induced by hyaluronidase as an acute response to adrenocortical extract. Similarly, Reppert, Donegan, and Hines[16] noted the inhibitory effect of ascorbic acid on dye spread in rabbits and suggested that the hyaluronidase-hyaluronic acid system was influenced by this vitamin. Further work is required on this.

## SUMMARY

1. Female guinea pigs were sensitized with horse serum. Histamine response was measured using pontamine sky blue.

2. Sensitization increased the histamine response.

3. Sodium loading and depletion reduced the response after sensitization but did not alter the findings in normal animals.

4. The histamine response in sensitized animals was reduced by hydrocortisone, ascorbic acid, and anti-hyaluronidase, and antiserotonin and d-lysergic acid diethylamide tartrate. These findings are discussed.

I am grateful to Professor R. J. S. McDowall for stimulating the early part of this work and to Professor R. J. Brocklehurst for reading the manuscript. My thanks are also due to Mr. A. D. B. Chant for technical assistance.

REFERENCES

1. Brocklehurst, W. E., Humphrey, J. H., and Perry, W. L. M.: The Role of Histamine in Cutaneous Antigen-Antibody Reactions in the Rat, J. Physiol. **129:** 205-224, 1955.
2. Buxton, R. St. J., and McDowall, R. J. S.: Effect of Sensitization on the Electrolyte Composition of Certain Tissues in the Guinea Pig, J. Physiol. **147:** 109-114, 1959.
3. Claude, A., and Duran-Reynals, F.: On the Existence of a Factor Increasing Tissue Permeability in Organs Other Than the Testicle, J. Exper. Med. **60:** 457-462, 1934.
4. Feldberg, W., and Miles, A. A.: Regional Variations of Increased Permeability of Skin Capillaries Induced by a Histamine Liberator and Their Relation to the Histamine Content of the Skin, J. Physiol. **120:** 205-213, 1953.
5. Fink, M. A.: Anaphylaxis in the Mouse: Possible Relation of the Schultz-Dale Reaction to Serotonin Release, Proc. Soc. Exper. Biol. & Med. **92:** 673-675, 1956.
6. Fischer, P., and Lecomte, J.: Hyperexcrètion urinaire d'acide 5-hydroxyindole-acètique au cours du choc anaphylactique, Compt. rend. soc. biol. **150:** 1649-1650, 1956.
7. Hayes, M. A., and Baker, B. L.: The Effect of Parenterally Administered Adrenocortical Extract on the Intradermal Spreading Action of Hyalurondiase, Endocrinology **49:** 379-383, 1951.
8. Kellaway, C. H., and Cowell, S. J.: On the Concentration of the Blood and the Effects of Histamine in Adrenal Insufficiency, J. Physiol. **57:** 82-99, 1922.
9. Miles, A. A., and Miles, E. M.: Vascular Reactions to Histamine, Histamine Liberator and Leukotoxine in the Skin of Guinea Pigs, J. Physiol. **118:** 228-257, 1952.
10. Miles, A. A.: Unpublished work cited by Feldberg and Miles.[4]
11. Parratt, J. R., and West, G. B.: 5-Hydroxytryptamine and the Anaphylactoid Reaction in the Rat, J. Physiol. **139:** 27-41, 1957.
12. Perla, D.: Effect of Ascorbic Acid on Resistance of Suprarenalectomized Rats to Histamine, Proc. Soc. Exper. Biol. **35:** 390-391, 1936.
13. Perla, D., and Marmorston, J.: Role of Vitamin C in Resistance, Arch. Path. **23:** 543-575 and 683-712, 1937.
14. Perry, W. L. M.: Unpublished work reported by Brocklehurst et al.[1]
15. Perry, W. L. M.: Ciba Foundation Symposium on Histamine, London, 1956, J. & A. Churchill, Ltd.
16. Reppert, E., Donegan, J., and Hines, L. E.: Ascorbic Acid and the Hyaluronidase-Hyaluronic Acid Reaction, Proc. Soc. Exper. Biol. **77:** 318-320, 1951.
17. Riley, J. F., and West, G. B.: Skin Histamine: Its Location in the Tissue Mast Cells, Arch. Dermat. Syph. (Chicago) **74:** 471-478, 1956.
18. Seifter, J., Baeder, D. H., Begany, A. J., Rosenkranz, G., Djerassi, C., Pataki, J., and Kaufman, S.: Method of Screening Steroids on Synovial Membrane for Possible Antiarthritic Action, Fed. Proc. **9:** 314, 1950.
19. Sudak, F. N., Wyman, L. C., and Fulton, G. P.: Effect of Histamine on Electrocardiograms and Serum Electrolytes of Intact, Cortisone Treated and Adrenalectomized Hamsters, Am. J. Physiol. **194:** 53-56, 1958.
20. Yannet, H., and Darrow, D. C.: The Effects of Depletion of Extracellular Electrolytes on the Chemical Composition of Skeletal Muscle, Liver and Cardiac Muscle, J. Biol. Chem. **134:** 721-737, 1940.

# EVALUATION OF THE STEROID TREATMENT OF ASTHMA SINCE 1950

*Horace S. Baldwin, M.D.,* Murray Dworetzky, M.D.,** and Norman J. Isaacs, M.D.,****
*New York, N. Y.*

THE USE of the corticosteroids in asthma presents problems that are unique to the disease and peculiarly related to current questions as to the dangers of steroid therapy. In the first place, asthma in a high percentage of patients is reversible when the sum total of stimuli to the asthmatic state are studied and treated. Also, in intractable cases, chronic infection in the respiratory tract is so common that steroid treatment carries with it the dangerous possibility of lowering the patient's resistance to infection[1] and masking the development of acute intercurrent infection, especially pneumonitis. Therefore the physician, when starting an asthmatic patient on steroid therapy, should be sure of the thoroughness of his analysis of the patient's asthmatic factors so that if steroid therapy is instituted the dosage will be at the minimum effective amount. At all times the hazards of steroid therapy should be kept in mind but with the added precaution to be alert to the problems of respiratory infections that are so common in the asthmatic patient. The present paper is based on a study of forty-eight patients with continuous intractable asthma and thirty-nine patients with intermittent severe asthma in whom corticosteroids have been used. The investigation began in 1950 when cortisone was first used in The New York Hospital as an adjunct in the treatment of asthma and includes experience with the various steroid compounds, prednisone, 6 methyl prednisolone, triamcinolone, and dexamethasone.[2-5] The study presents data on the general conditions of patients in the Continuous and Intermittent groups, the duration and dosage of steroids used, side effects and complications, mortality, and analysis of cases where steroid therapy was unsuccessful, the management of interval surgery, hospitalization before and after steroid treatment, and a summary of ancillary medical treatment with particular emphasis on management of chronic and acute infection.

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From the New York Hospital-Cornell Medical Center, Department of Medicine, New York, N. Y.

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*Associate Attending Physician, The New York Hospital; Associate Professor of Clinical Medicine, Cornell University Medical College.

**Assistant Attending Physician, The New York Hospital; Assistant Professor of Clinical Medicine, Cornell University Medical College.

***Physician to the Outpatient Department, The New York Hospital. Instructor in Medicine, Cornell University Medical College.

GENERAL CONDITIONS OF PATIENTS STUDIED

An analysis was made of the contributory factors in both the Continuous and Intermittent groups of patients (Table I). Allergic factors comprising sensitivity to various inhalants were established by history and confirmed by intradermal skin testing and were found in thirty-nine patients of the Continuous group (81 per cent) and 34 patients of the Intermittent group (85 per cent). Infectious factors comprised infection in the nasal sinuses, pharyngeal lymphoid tissue, and in the lungs and bronchi. They were established by rhinological examination, cultures of the sputum, nasal secretions, and sinus washings and by x-ray examination of the paranasal sinuses and chest. In the Continuous group forty-one patients (85 per cent) had evidence of infectious factors as compared with twenty-four (61 per cent) in the Intermittent group. Psychosomatic factors precipitating or augmenting the asthmatic reactions were evaluated in all patients but it is difficult to state with certainty whether these factors were secondary to the chronic asthma problem or were initiating factors. Psychosomatic factors were considered important in contributing to the asthma in twenty-two patients (45 per cent) of the Continuous group compared with 7 patients (18 per cent) in the Intermittent group. Irreversible structural changes involving the pulmonary-cardiac system and indicating chronic pulmonary fibrosis and emphysema were determined by physical examination, respiratory function tests, and x-ray examination.

TABLE I. GENERAL CONDITIONS OF PATIENTS STUDIED

| | CONTINUOUS (48) | INTERMITTENT (39) |
|---|---|---|
| Age of onset (average and range) | 32.2 yr. (1-56) | 29.4 yr. (1-91) |
| Age beginning steroids (average and range) | 45.5 yr. (6-76) | 44.2 yr. (5-91) |
| Contributory Factors | | |
| 1. Allergic | 39 patients (81%) | 34 patients (85%) |
| 2. Infectious | 41 patients (85%) | 24 patients (61%) |
| 3. Psychosomatic | 22 patients (45%) | 7 patients (18%) |
| 4. Structural | 24 patients (50%) | 5 patients (15%) |

Structural changes of this sort were found in twenty-four patients (50 per cent) of the Continuous group compared with five patients (15 per cent) in the Intermittent group. It will be readily observed that whereas allergic factors are about equal in both groups, infectious and psychosomatic factors are definitely more a problem in the group requiring continuous steroid therapy, and coincidentally the amount of irreversible structural changes in the pulmonary-cardiac system is greater in the Continuous group than in the Intermittent group.

DURATION OF STEROID TREATMENT

Continuous steroid therapy has been maintained in forty-eight patients, ranging from one to nine years' duration (Table II). The average duration

has been 4.4 years. In the Intermittent group of thirty-nine patients, treatment has ranged from a few days to a maximum of 210 days, with an average of forty days' duration. In this group, a total of eighty-four courses of steroid therapy has been given to the thirty-nine patients.

TABLE II. DURATION OF STEROID THERAPY IN 48 PATIENTS TREATED CONTINUOUSLY

| YEARS | PATIENTS |
|---|---|
| 0-1 | 4 |
| 1-2 | 9 |
| 2-3 | 5 |
| 3-4 | 5 |
| 4-5 | 7 |
| 5-6 | 3 |
| 6-7 | 6 |
| 7-8 | 5 |
| 8-9 | 3 |
| 9-10 | 1 |

### DATA ON THE VARIOUS STEROIDS USED

Many of the patients in both groups (Table III) received several of the various steroids to evaluate comparative clinical effect and dosage. Since it became apparent early in the study that prednisone had distinct advantages over cortisone and hydrocortisone in causing less sodium retention and potassium excretion, these were discontinued from further use. The largest number of patients received prednisone because it was available earlier than 6 methyl prednisolone, triamcinolone, and dexamethasone.

TABLE III. MILLIGRAMS PER DAY OF VARIOUS STEROID COMPOUNDS USED

| | CONTINUOUS | | | INTERMITTENT | |
|---|---|---|---|---|---|
| | PATIENTS | DAILY DOSE (MG.) | MEAN & RANGE (MG.) | PATIENTS | (DOSE RANGE) (MG.) |
| Cortisone | 18 | 51.3 | (12.5-300) | 9 | (2.5-100) |
| Hydrocortisone | 19 | 39.5 | (10-160) | 6 | (1.5-100) |
| Prednisone | 43 | 9.8 | ( 2.5-90) | 26 | (1.5-30) |
| 6 methyl prednisolone | 25 | 7.6 | ( 2.0-6.0) | 14 | (4-20) |
| Triamcinolone | 18 | 7.0 | ( 2.0-24) | 6 | (4-32) |
| Dexamethasone | 24 | .89 | ( 1.125-4.5) | 8 | (1.0-3.0) |

In general, the average daily maintenance dosage of steroid of cortisone (51.3 mg.), hydrocortisone (39.5 mg.), prednisone (9.8 mg.), 6 methyl prednisolone (7.6 mg.), triamcinolone (7 mg.), and dexamethasone (0.89 mg.) falls into the rated milligram conversion dosage of one steroid to another. However, there was a wide range of daily dosage necessary to secure satisfactory clinical results since certain patients under the stress of increased precipitating factors required heavier dosage, whereas, at times, when a minimum of such factors was operating, only a small maintenance dose was required.

### COMPLICATIONS AND SIDE EFFECTS

It is important to emphasize that the steroid compounds can produce profound metabolic effects and that serious complications have been reported with their use.[6, 7] In the group of patients reported here there have been

no fractures, hemorrhages, or psychoses. No cases of diabetes or gastrointestinal ulcers have developed subsequent to steroid treatment. One patient with diabetes required larger insulin dosage and more rigid adherence to diet. A patient with a diagnosis of duodenal ulcer prior to steroid treatment has been on steroids for the past five years with no symptomatic or x-ray evidence of activation of the ulcer. Three patients had hypertension before the use of steroids but were not made worse with them. One patient lost considerable weight and had muscle wasting after several months' treatment with triamcinolone. He quickly gained his normal weight and muscular development after being shifted to prednisone. One patient receiving dexamethasone developed purpuric spots.

Cushing facies, mild edema, hirsutism, pronounced appetite, acne, palpitation, insomnia, and weakness have occurred in several patients but soon disappeared when the amount of steroid was adjusted or another compound was substituted.

The incidence and extent of osteoporosis are difficult to evaluate but no fractures have occurred. Fourteen patients had periodic x-ray examination of the bones after being placed on steroids for variable lengths of time, seven of whom had no evidence of osteoporosis. These included four males and three females from thirty-nine to fifty-five years of age who, with one exception, had been on steroids from two to eight years. The remaining seven patients, four females and three males, showed osteoporosis. Six of these varied in age from fifty-one to sixty-one, the other being thirty-two years of age. All had been on steroids from two to five years. Four of these were placed on an anti-osteoporotic regimen. Subsequent roentgenograms showed an increase in osteoporosis in only one of the seven.

In general, fewer side effects have been noted in the patients taking prednisone and 6 methyl prednisolone than with triamcinolone and dexamethasone. In all cases the dosage of steroid has been cut to the minimum effective amount as soon as possible and this, in the great majority of cases, has been small. This fact probably explains the few complications and side effects of steroid therapy that have occurred in this series.

### MORTALITY

In this series of eighty-seven patients, six have died. Two males died of acute coronary thrombosis at the ages of forty-nine and fifty-seven, one male (aged sixty-eight) died of osteogenic sarcoma, one male (aged seventy) from bronchogenic carcinoma, one female (aged 93) from generalized arteriosclerosis and senility, and one female (aged 52) after withdrawal of all steroid medication while away from our supervision. In none of these cases was there any obvious connection between death and steroid medication except in the last case mentioned.

### FAILURE OF STEROID THERAPY

Failure of steroid therapy to produce symptomatic relief of the asthma has been observed in eight patients. In seven patients, inability to clear up

infectious foci in the respiratory tract and irreversible structural changes in the lungs have appeared to be the reasons for failure. In one patient, failure of steroid therapy was attributed to poor cooperation and strong psychological factors.

Two of the cases were in the Intermittent group. One of these, a woman of sixty-two years of age, had had asthma for ten years. The administration of 16 mg. of triamcinolone daily for one week failed to improve the acute asthma until infected antra were irrigated and drainage established. Coincident with this procedure, the asthma improved and steroids were discontinued. The other case in this group was a girl, five years of age, treated with 6 methyl prednisolone for three weeks with 4 to 12 mg. per day. No improvement resulted. Eventually the asthma was controlled with removal of infected adenoids and no further steroid medication has been necessary.

The failures in the Continuous group can be briefly summarized as follows:

CASE 1.—A woman, aged fifty-two, had had asthma for thirty years; equivocal allergy to pollens and dust. There was a chronic nasal sinus infection with polyps and marked psychological stress. Steroid therapy had been started at the age of forty-four which had resulted in dramatic control for seven years. There were gradually more frequent pulmonary infections which were less susceptible to various antibiotics. She was treated with cortisone, hydrocortisone, prednisone, 6 methyl prednisolone, and triamcinolone. In the eighth year of steroid treatment, the respiratory infection could no longer be controlled. Steroid treatment was not clinically effective and she was referred to a hospital for chronic care. One year later she died in another hospital in New York. Autopsy showed no abnormal findings in the pituitary or adrenal glands and no cause of death was found in other organs. It is noteworthy that steroids which she had been taking continuously for over eight years had been discontinued completely one week before death.

CASE 7.—A woman, aged fifty-two had asthma which had started at the age of forty-four. There were infectious factors present in the nasal sinuses, bronchiectasis, considerable pulmonary emphysema, but no allergic factors. Psychological factors were not prominent. She was given an average dose of prednisone, 20 mg. per day, for six months without improvement; the failure was attributed to emphysema and inability to control infection from bronchiectasis and sinusitis.

CASE 13.—A woman, aged forty-two. Hayfever had started at the age of thirty-eight, and asthma at the age of forty. The allergic factors in the asthma were pollen and house dust. They were infectious factors present in the nasal sinuses and bronchi. The patient was given prednisone, 15 to 50 mg., per day for over thirteen months with no definite improvement; the failure was attributed to inability to control infectious factors.

CASE 18.—A woman, aged twenty-three, had asthma which had started at the age of twelve. There was no definite allergic factors by history or skin testing. Chronic hyperplastic sinusitis and polyps were present, as well as marked psychogenic factors. In spite of nasal surgery, antibiotics, and continuous steroid therapy for five years, the asthma remains intractable, with serious flare-ups of pneumonitis and acute episodes of status asthmaticus. This failure was attributed to inability to clear up chronic respiratory infection as well as strong emotional factors.

CASE 23.—A woman, aged forty-six, had had asthma which had started at the age of forty-five. She had severe diabetes which required insulin treatment. The patient was allergic to pollens and animal danders and had marked psychological factors. There was no respiratory infection or structural changes. She was treated irregularly with prednisone and 6 methyl prednisolone, up to 20 mg. of each per day, for three months. She refused

to eliminate her pet or be immunized to pollens. She was also fearful of steroids. The failure of steroid therapy was attributed to poor cooperation and strong psychological factors.

CASE 34.—A boy, aged sixteen had had asthma since one year of age. He was allergic to pollens and animal danders. There was chronic infection present in tonsils and adenoids and marked emphysema. Psychological factors were moderate. Tonsils and adenoids were removed and allergic management instituted. He was treated with antibiotics, ancillary medication, prednisone, and 6 methyl prednisolone, up to 20 mg. per day, for nearly two years with no particular effect. The steroids were discontinued but the other regimens were not. The asthma has gradually improved.

### SURGICAL COMPLICATIONS

One of the most important complications of steroid therapy is the danger of adrenal failure accompanying operation.[8, 9] In this series we have carefully followed the usual precautions and given supplementary steroids before and after operation and have had no complications of this sort. There have been three children who have had tonsils and adenoids removed, ten patients have had the Caldwell-Luc operation, one a partial thyroidectomy for the removal of a cyst, one a cervical laminectomy for the removal of a spur, one a cholecystectomy. Two patients have had normal deliveries while on continuous steroid therapy.

### HOSPITAL RECORD BEFORE AND AFTER STEROID TREATMENT

In the Continuous group, twenty-eight patients (58 per cent) required one to seven hospitalizations prior to steroid therapy. After the use of steroids, sixteen of the twenty-eight required one to four additional hospitalizations. Only one patient who was not hospitalized prior to the use of steroids required hospitalization subsequently.

In the Intermittent group, five patients (13 per cent) required hospitalization before steroids. Subsequently none has required hospital care.

### SPECIFIC AND ANCILLARY MANAGEMENT

The importance of conventional, standard management of the asthmatic patient under steroid therapy cannot be overemphasized.[10] Indeed, the Continuous group consisted of those patients in whom all other forms of conventional therapy, including specific management (consisting of elimination of allergens and immunization) and nonspecific management (including bronchodilators, expectorants, antimicrobials, and nasal surgery where indicated), had failed to give relief. The patients in the Intermittent group were those, for the most part, in whom attacks of acute asthma were due either to infection or exposure to allergens, often complicated by psychogenic factors. In these patients, steroids were used to control the acute episodes until such time as conventional therapy was adequate to maintain relief of symptoms.

Bronchodilators and expectorants were used to varying degrees in all patients in both groups. Specific therapy consisting of elimination of allergens

and immunization with allergenic extracts was employed in most patients in both groups (Table IV). It is interesting to note that antimicrobial therapy was employed at one time or another with about the same frequency as specific therapy. Antimicrobials were used many times in most patients whenever there was clinical or laboratory evidence of respiratory infection and approximately the same proportion of patients in the Continuous and Intermittent groups received antimicrobial therapy throughout the period of study (Table IV).

TABLE IV. SPECIFIC AND ANCILLARY MANAGEMENT BEFORE AND AFTER STEROID THERAPY

| | CONTINUOUS GROUP (48) | | | INTERMITTENT GROUP (39) | | |
|---|---|---|---|---|---|---|
| | BEFORE | AFTER | TOTAL | BEFORE | AFTER | TOTAL |
| Specific | 29 | 42 | 45 | 29 | 34 | 36 |
| Antimicrobials | 28 | 38 | 43 | 24 | 30 | 35 |
| Nasal surgery | 19 | 16 | 26 | 11 | 1 | 12 |

Nasal surgery for the purpose of eliminating foci of infection or promoting better aeration and drainage was performed in a total of twenty-six of the forty-eight patients in the Continuous group and in twelve of the thirty-nine patients in the Intermittent group (Tables IV and V). More patients in the Continuous group were subjected to nasal operation than those in the Intermittent group. This difference was most striking in the incidence of operation after steroids were begun, with sixteen patients in the Continuous group having nasal operation in contrast to only one in the Intermittent group. Since there was no significant difference between the two groups, regarding the incidence of infectious factors, it is most likely that the difference in the incidence of nasal surgery is a reflection of the greater severity of asthma in the Continuous group in which more aggressive therapy was justified.

TABLE V. NASAL SURGERY BEFORE AND AFTER STEROID THERAPY

| | CONTINUOUS GROUP (48) | | | INTERMITTENT GROUP (39) | | |
|---|---|---|---|---|---|---|
| | BEFORE | AFTER | TOTAL | BEFORE | AFTER | TOTAL |
| Caldwell-Luc and revisions | 8 (inc. 1 rev.) | 9 (inc. 2 rev.) | 12 | 1 | 0 | 1 |
| Polypectomy | 11 | 7 | 14 | 4 | 0 | 4 |
| Submucous resection | 5 | 1 | 6 | 2 | 1 | 3 |
| Ethmoidectomy | 2 | 2 | 4 | 2 | 0 | 2 |
| Nasal plastic | 0 | 0 | 0 | 1 | 0 | 1 |
| Antrotomy | 0 | 0 | 0 | 2 | 0 | 2 |
| Tonsilectomy and adenordectomy | 3 | 3 | 5 | 2 | 0 | 2 |
| Total number of patients | 19 | 16 | 26 | 11 | 1 | 12 |

The details of nasal surgery performed in these patients are tabulated in Table V. Eight of the forty-eight patients in the Continuous group had the Caldwell-Luc procedures before beginning on steroids; one of these required a revision of the procedure. An additional nine patients had Caldwell-Luc procedures after the steroids were begun, including two revisions. In contrast, only one patient in the Intermittent group had a Caldwell-Luc procedure and this one was prior to steroid therapy. Since polypectomies are generally done

whenever polyps are found, it is reasonable to assume that polyps were present in a much greater number in the Continuous group than in the Intermittent. Eighteen of the forty-eight patients in the Continuous group had polypectomies, eleven before and seven after steroids were begun. All had a single polypectomy except four, one of whom had thirteen, one had five, and two, two each. In these four patients, polypectomies were of equal frequency, before and after steroid therapy was begun.

Unlike frequency of Caldwell-Luc procedures and polypectomies, submucous resections and ethmoidectomies were performed in about the same proportion of patients in the Continuous and Intermittent groups.

Examination of the data on specific and ancillary management before and after steroid therapy makes it apparent that steroid treatment did not eliminate the necessity of standard, conventional management. Unless the required ancillary measures are continued, larger doses of steroids are necessary, thereby increasing their hazards.

## DISCUSSION

One of the most important questions involved in steroid treatment of the patient with asthma is whether or not the initiation of steroids means permanent reliance on those drugs with an increase in dosage as time goes on. An evaluation of the Intermittent group indicates that there are numerous patients in whom steroid therapy can be limited to short-term periods until the asthmatic factors are controlled; at this time steroid therapy can be terminated. In the Continuous group, which comprises those patients with intractable asthma usually with multiple asthmatic factors consisting of allergic stimuli, chronic respiratory infection, psychosomatic tendencies, and structural changes, it would seem from our experience that, if the cooperation of the patient can be obtained toward elimination of and immunization to allergic factors, if chronic respiratory infection can be controlled by elimination of foci of infection by suitable operation and the use of antibiotics, and if structural changes in the pulmonary and cardiac areas have not progressed too far, the daily minimum effective dose of steroids can be gradually reduced and, at times, eliminated altogether. The psychological factors, which play such an important part in precipitating and augmenting the asthmatic state, are often relieved when, through effective use of steroids, the asthmatic patient is able to resume his job or pursue her usual domestic duties with the insecurity attached to constant asthma gone. When ACTH and cortisone were first used successfully in relieving asthma, hope arose that by their use the fundamental asthmatic response "might be favorably altered to the wide variety of stimuli which are the active cause of the disease syndrome and the irreversible structural changes which may result."[2] However, in the series of cases reported here, unless the asthmatic factors are controlled, steroid treatment cannot be discarded without the return of asthmatic symptoms.

In reviewing this series of patients we have been continually impressed with the importance and frequency of respiratory infection. Failure to secure

satisfactory results with the steroids has often appeared to be due to inability to control infection in spite of operation to remove infected foci and the frequent use of antibiotics. One of the great problems of steroid therapy consists in its effect on infection and the possibility that it may suppress the protective inflammatory response, as cited by Germuth,[11] and thus favor the development of subclinical infection into active infection. Our experience indicates the value of the frequent use of antibiotics in these cases but, by the same token, the alert physician must be aware of the dangerous effects of antibiotics in developing resistant strains of bacteria, especially the staphylococcus, and the possibility of overcoming bacterial infection but favoring the development of pseudomona and fungal infections. There is increasing evidence that most of our acute respiratory infections today are viral in origin rather than bacterial.[12] At present it would appear that the effects of steroids and antibiotics in the treatment of viral infections have not been definitely established. On the other hand, where chronic pulmonary disease exists, the safer course is to treat acute intercurrent infections with antibiotics.

Finally, we would emphasize that the great majority of patients with asthma need never take steroids if they are properly diagnosed and treated specifically, and with the conventional ancillary drugs. On the other hand, where conventional methods are not adequate, the addition of the steroid compound in minimal effective amounts represents a most important step forward in the management of allergic states and especially in the case of intractable asthma where their use often enables the patient to return to a useful and comfortable life.

## SUMMARY AND CONCLUSIONS

1. Eighty-seven patients with asthma have been treated on a continuous or intermittent basis with various steroids consisting of cortisone, hydrocortisone, prednisone, 6 methyl prednisolone, triamcinolone, and dexamethasone. Continuous therapy has averaged 4.4 years. Intermittent therapy has ranged from a few days to 210 days.

2. Those patients requiring continuous steroid treatment had a higher incidence of infectious and psychosomatic factors than those in the intermittent group. Allergy to specific inhalants was about equal in both groups.

3. The average daily maintenance dose of the various steroid compounds falls into the generally accepted milligram conversion dosage of one steroid to another.

4. No serious metabolic side effects attributable to steroid treatment were noted but it should be emphasized that the dosage of steroids was the minimum effective amount and carefully supervised.

5. During the ten years of this investigation, six of the patients have died. One patient died after abrupt cessation of steroid medication while away from our supervision. In none of the other cases has there been any obvious connection between death and steroid therapy.

6. Eight patients have failed to show satisfactory clinical response to steroid treatment. Failures were chiefly associated with chronic respiratory infection, inability to clear up infectious foci, and irreversible structural changes in the lungs. The tendency of steroids to mask infection and suppress protective inflammatory response is emphasized as a serious hazard in the steroid treatment of the asthmatic patient.

7. There have been no instances of adrenal shock accompanying operation in this series. Supplementary steroid medication has been given before and after surgical procedures.

8. The number of hospital admissions per patient following institution of steroid therapy has been decreased.

9. Steroids have an important place in the treatment of severe intractable asthma restoring many patients who are incapacitated to a fairly normal life in so far as structural changes permit. Because of the dangers of steroid medication, dosage should be kept at a minimum, carefully supervised, and terminated as soon as possible through control of the individual asthmatic factors and ancillary medication.

REFERENCES

1. Romansky, M. J.: Steroid Therapy in Systemic Infections. (Report to the Council on Drugs), J. A. M. A. **170:** 1179, 1959.
2. Baldwin, H. S., and DeGara, P. F.: Hospital and Ambulatory Treatment of Asthma and Eczema With ACTH and Cortisone, J. ALLERGY **23:** 15, 1952.
3. Baldwin, H. S., DeGara, P. F., Spielman, A. D., and Dworetzky, M.: ACTH and Cortisone in the Treatment of Asthma, J. ALLERGY **26:** 44, 1955.
4. Irwin, J. W., and Burrage, W. S.: The Role of Adrenocortical Steroids in the Treatment of Intractable Bronchial Asthma, J. ALLERGY **29:** 233, 1958.
5. Schwartz, E.: Steroids in Treatment of Allergic Disease, New York J. Med. 151, 1957.
6. Thorn, G. W., and Jenkins, D.: Diseases of the Adrenal Cortex *in* Principles of Internal Medicine, New York, 1958, McGraw-Hill Book Company, vol. 1, p. 579.
7. Editorial: Late Effects of Cortisone and Corticotropin J. ALLERGY **25:** 190, 1954.
8. Salassa, R. M., Bennett, W. A., Keating, F. R., Jr., and Sprague, R. G.: Postoperative Adrenal Cortical Insufficiency. Occurrence in Patients Previously Treated With Cortisone, J. A. M. A. **152:** 1509, 1953.
9. Schneewind, J. H., and Cole, W. H.: Steroid Therapy in Surgical Patients. (Report to the Council on Drugs), J. A. M. A. **170:** 1411, 1959.
10. Segal, M. S.: Current Status of Therapy in Bronchial Asthma. (Report to the Council on Drugs), J. A. M. A. **169:** 1063, 1959.
11. Germuth, F. G., Jr.: The Role of Adrenocortical Steroids in Infection, Immunity and Hypersensitivity, Pharmacol. Rev. **8:** 1, 1956.
12. McDermott, W., Kilbourne, E. D., Rogers, D. E., and Rose, H. M.: Influenza and Upper Respiratory Infections, Bull. New York Acad. Med. **36:** 22, 1960.

# IMMUNOLOGIC REACTIONS TO PENICILLIN

*Jean Harris, M.D.,* and John H. Vaughan, M.D., Rochester, N. Y.*

HYPERSENSITIVITY to penicillin has been observed during recent years in increasing frequency. Penicillin skin tests have proved to be of value primarily in identifying some of the individuals liable to immediate anaphylaxis. Presence or absence of skin reactivity has not, however, been of prognostic value in respect to later development of serum sickness, delayed urticaria, or other skin eruptions. In 1958 Ley and collaborators[1] reported that penicillin-coated erythrocytes were agglutinated by the sera of some individuals who have been treated with penicillin. He did not relate the hemagglutinin to skin sensitizing antibody, nor did he particularly implicate it in sensitivity reactions. Nevertheless, it is our opinion that he might have provided a new tool useful for the investigation of penicillin hypersensitivity. Therefore, we have used the hemagglutination test to examine the sera of patients observed to have had penicillin reactions. Comparison was made with the sera of patients recently treated with penicillin, but suffering no reactions, and with the sera of random patients with various other diseases.

## METHODS

*Preparation of Cells.*—Fifty milliliters of blood was collected from a type "O" donor and added to 12 ml. of ACD† solution. The red cells were "penicillinized" by adding 6 ml. of the citrated blood to 200,000 units of penicillin G powder. This was incubated 1 hour at 37° C. and stored in the refrigerator. Portions of the penicillinized cells were removed, washed three times with pH 7.3 phosphate buffered saline, and re-suspended as 2 per cent or 5 per cent cell suspensions. The 2 per cent cell suspension was used for screening tests. The 5 per cent suspension was used in titrations in which the penicillinized cells were subdivided into aliquots after exposure to antibody, preparatory to differential Coombs tests. Control suspensions of washed "O" cells which had not been exposed to penicillin were employed in all experiments.

From the University of Rochester School of Medicine and Dentistry, Rochester 20, N. Y.

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*U. S. Public Health Service Trainee of the National Institutes of Allergy and Infectious Diseases and a Postdoctoral Fellow of the National Institutes of Health (Present Address: Howard University Medical School, Washington, D. C.).

†Per 100 ml.: 1.47 Gm. dextrose; 1.32 Gm. sodium citrate; 440 mg. citric acid.

*Hemagglutination.*—As a screening procedure, 0.05 ml. of a 2 per cent cell suspension was added to 0.3 ml. of serum. The serum and cells were then incubated at room temperature for fifteen minutes with gentle agitation, centrifuged at 800 r. p. m. for one minute, and read directly for agglutination. The cells were then washed three times with normal saline and re-suspended in a modified "Coombs" serum having specificity either for human $\gamma_2$-globulin or for serum globulins other than $\gamma_2$-globulins.[2, 3] These then were allowed to stand at room temperature fifteen minutes, centrifuged gently, and read again for hemagglutination. The use of the "Coombs" serum was designed to detect instances in which antibodies were present, but in which the antibodies for quantitative or qualitative reasons failed to provide direct agglutination. In other instances, the Coombs technique was used to enhance agglutination already seen to some degree.

TABLE I. POSITIVE HEMAGGLUTINATION REACTIONS

| DIAGNOSIS | INCIDENCE |
|---|---|
| Group 1. "Probable" penicillin reactions | 6/16 |
| Group 2. "Suspected" penicillin reactions | 0/5 |
| Group 3. Rheumatic fever patients on oral penicillin | 3/307 |
| Group 4. Random ward subjects | 0/167 |
| Group 5. Patients recently completing a course of penicillin (off drug 2-7 days) | 0/32 |
| Group 6. Collagen-vascular disease | 4/13 |
| Total | 13/541 |

*Skin Testing.*—One to two hundredths milliliter of a freshly prepared solution containing 1,000 units of penicillin G per milliliter of saline was injected intracutaneously. A control site was injected with an equal amount of saline. Both sites were read at the end of fifteen to twenty minutes. If no reaction was observed, 0.01 ml. of a solution containing 10,000 units penicillin G per milliliter was injected intracutaneously and the skin again observed for fifteen to twenty minutes. If the reactions were still negative, the sites were again read at the end of twenty-four hours. Patch tests were performed with an ointment containing 100,000 units penicillin G powder per gram of water-miscible base. Tested areas were observed at intervals of twenty-four and forty-eight hours.

## RESULTS

*Clinical Observations.*—Of 541 serum samples examined, 13 gave positive hemagglutinin patterns: six of sixteen patients with a diagnosis of "probable" penicillin reaction, three of 307 rheumatic fever patients on oral penicillin prophylaxis, and four of thirteen patients with "collagen-vascular" disease. None of 167 random ward subjects, none of thirty-two selected ward patients recently completing courses of penicillin, and none of five patients "suspected" of having had a penicillin reaction had positive reactions. Separation of patients into "probable" and "suspected" categories of penicillin reactions was necessitated by the obvious difficulty of establishing definite clinical

diagnoses in patients who often were receiving more than one drug, and in whom the adverse clinical picture might also have represented an unusual expression of the underlying disease rather than a drug reaction.

Thirteen of the sixteen individuals experiencing probable penicillin reactions (Group 1) were also skin tested. A comparison of these skin test results with the penicillin hemagglutination test was made (Table II). Of four individuals with an erythematous macular rash, one gave an immediate skin reaction and another gave a delayed erythema in twenty-four hours.

TABLE II. "PROBABLE" PENICILLIN REACTIONS

| CLINICAL PICTURE | NUMBER OF PATIENTS | POSITIVE HEMAGGLUTINATION | POSITIVE SKIN TEST | | | TOTAL POSITIVE |
|---|---|---|---|---|---|---|
| | | | IMMED. | 24 HR. | PATCH | |
| Erythematosus macular rash | 6 | 1/6 | 1/4 | 1/4 | 0/1 | 3/6 |
| Exfoliative dermatitis | 3 | 2/3 | 1/2 | 1/2 | | 3/3 |
| Urticaria | 4 | 1/4 | 3/4 | 1/4 | 0/4 | 4/4 |
| Angioneurotic edema | 1 | 0/1 | 0/1 | 1/1 | 0/1 | 1/1 |
| Serum sickness* | 2 | 2/2 | 1/2 | 0/2 | 0/2 | 2/2 |
| Totals | 16 | 6/16 | 6/13 | 4/13 | 0/8 | 13/16 |

*Fever, arthralgia, lymphadenopathy, and urticaria.

Neither of these two individuals had the one positive hemagglutinin reaction seen in this subgroup. One of the three individuals with exfoliative dermatitis had a positive immediate skin reaction and also gave a positive hemagglutinin reaction. Another with exfoliative dermatitis showed delayed erythema and edema in skin testing and was negative in the hemagglutinin test. Three of four individuals with urticaria gave positive immediate skin reactions; the fourth gave a delayed (twenty-four hour) erythema and edema. Only one of these four patients gave positive hemagglutination. The one patient with angioneurotic edema gave only a delayed erythema on skin test. One of two patients with serum sickness gave a wheal and flare when skin tested shortly after her initial episode. The other patient, although negative by skin test immediately after her reaction, was positive with a strong wheal and flare response two months later. Both patients with serum sickness had positive hemagglutination reactions.

TABLE III. HEMAGGLUTINATION VS SKIN TESTS

| HEMAGGLUTINATION | | SKIN TEST POSITIVE | |
|---|---|---|---|
| | | IMMEDIATE | DELAYED |
| Positive | 6 | 3 | 0 |
| Negative | 10 | 4 | 4 |

By the combined use of skin testing and serum hemagglutination, thirteen of the sixteen patients with probable penicillin reactions were shown to have developed an immunologic response to the drug. Of 8 patch tests performed, none gave a positive reading. The lack of identity of positive skin reactivity with presence of penicillin hemagglutinin is emphasized in Table III. Only

half the patients with demonstrable hemagglutinin also had positive skin tests, and positive skin tests were seen in the absence of hemagglutination.[1]

Penicillin hemagglutination was occasionally found in the sera of individuals who had been treated with penicillin but who had not been thought to have had a sensitivity reaction. The strengths of these hemagglutination reactions were generally less than those seen in the sera of patients diagnosed as having experienced penicillin reactions. The three patients with rheumatic fever on prophylactic penicillin were in this category. On review of their records, there was nothing to suggest a hypersensitivity reaction, either by the occurrence of skin eruptions or by the development of joint complaints, fever, or other symptoms consistent either with serum sickness or recurrent rheumatic activity.

Originally only the first five groups of patients were designated in this study. Group 6, "the collagen-vascular diseases," was established by virtue of the findings in two of our cases. The first was a 21-year-old girl, who had had a past history of vague arthralgias and suggestive blanching of the hands on exposure to cold, and who developed a blotchy, erythematous, infarctive skin lesion after a course of 10,000,000 units of penicillin given because of the incidental finding of a positive serological test for syphilis. Tests for treponema immobilizing antibody, however, were negative on repeated occasions. A very strong penicillin hemagglutination titer was noted some two months after the penicillin therapy and diminished slowly in titer for over the subsequent year. Angiitic skin lesions, demonstrated by biopsy, persisted in undiminished form during the year. The L.E. cell test was negative, but the diagnosis of lupus erythematosus has been strongly considered. It was felt that the development of the immune reaction to penicillin probably contributed significantly to the adverse course of vasculitis in this patient, but that the penicillin reaction was not the primary cause of the vasculitis. This patient had originally been classified in Group 4 of Table I, the "penicillin reactors." She was removed to Group 6 after recognition of the second patient, a 69-year-old woman with a clinical picture of rheumatoid arthritis, bronchopneumonia, and a positive L.E. cell test. This second patient had no clear-cut evidence of a penicillin reaction but had a positive hemagglutination reaction. She had been originally classified in Group 3 ("selected patients recently treated with penicillin").

Because of the interest engendered by these two latter patients, eleven sera from patients with established diagnoses of lupus erythematosus were taken from deep freeze storage and examined for penicillin antibodies. Two of these gave strong reactions. Review of the records of these patients revealed no definite clinical indications of penicillin reactions in them, although the lupus erythematosus was active in both prior to, during, and after the penicillin therapy. The eleven sera from patients with lupus erythematosus, together with the two above, have been grouped and constitute the thirteen sera of Group 6 of Table I.

*Laboratory Observations.*—Of the 13 sera positive for penicillin antibody, 12 demonstrated direct agglutination. Six were positive by "Coombs" test; in one of these the antibody was detected only by the "Coombs" technique.

Differential studies were performed with absorbed "Coombs" sera. One of the "Coombs" sera (anti-$\gamma_2$-globulin) had been prepared by injection of rabbits with $\gamma_2$-globulin (Fraction II-1,2) and absorbed with an agammaglobulinemic human serum. This serum reacted strongly with an Rh-positive cell coated with anti-Rh antibody, but failed to react with a cell coated with complement-fixing incomplete cold agglutinin.[2, 3] The other Coombs serum (anti-non-$\gamma_2$-globulin) was from a rabbit that had been injected with whole human serum and had been optimally absorbed with Fraction II-1,2. It failed to agglutinate Rh positive cells sensitized with anti-Rh antibody, but agglutinated strongly cells coated with incomplete cold agglutinin. Anti-non-$\gamma_2$-globulin sera have been shown to detect complement protein.[2, 3] These absorbed "Coombs" sera were used to test penicillinized cells that had been exposed to the patients' antibody and then rewashed three times in saline. Generally, any original strong agglutination of the penicillinized cells in a patient's serum was considerably reduced or obliterated by the washing procedure. Redevelopment of significantly strong agglutination was noted in the six sera indicated in Table IV. In three instances, agglutination reactions were noted in the anti-$\gamma_2$-globulin serum, consistent with the assumption that the penicillin hemagglutinin in these instances was a gamma globulin. In three other instances, agglutination developed in the anti-non-$\gamma_2$-globulin. Consistent with the assumption that these latter reactions depended upon complement fixation on the sensitized cell was the observation in one of the three (Johnson; Table IV) that hemagglutination activity was lost with aging and that the activity could be recovered by the addition to the aged serum of fresh normal serum containing no hemagglutinating antibody.

TABLE IV

| | DIRECT AGGLUTINATION | AGGLUTINATION ON RESUSPENDING THE WASHED CELLS IN: | | | | |
|---|---|---|---|---|---|---|
| | | | ANTI-$\gamma_2$-GLOBULIN | | ANTI-NON-$\gamma_2$-GLOBULIN | |
| | | SALINE | 1/50 | 1/200 | 1/20 | 1/100 |
| Knittle | +++ | ++ | +++ | +++ | + | + |
| DiBartolo | ++ | + | ++ | ++ | – | – |
| Levin | ++ | ± | +++ | +++ | – | – |
| Johnson | ++ | – | – | – | ++ | ++ |
| Siddons | +++ | ± | – | – | +++ | +++ |
| Rogoff | – | – | – | – | ++ | ++ |

The specificity of the penicillin hemagglutination reaction was well established by Ley in his original publication,[1] both by inhibition with penicillin and by the effect of penicillinase on the system. These same criteria for specificity were employed also in the present study. Even small amounts of penicillin added to test sera prior to addition of the penicillinized cells were capable of completely inhibiting the reaction of the sera with the cells (Table V), and penicillinase abolished the ability of penicillin to coat red cells for hemagglutination with these sera.

The synthetic penicillin, Syncillin (Penicillin-152), also could inhibit serum hemagglutination in some instances (Table V) quite as effectively as penicillin G. With some other sera, however, Syncillin was inactive in inhibition. In all inhibition studies, the cells were coated with penicillin G, and inhibition was assessed by preincubating the antibody-containing serum with the penicillin preparation. Failure of Syncillin to inhibit agglutination by a given serum thus would suggest that the antibody activity in that serum was predominantly for the side group (R-group, Fig. 1) rather that the basic penicillin radicle.

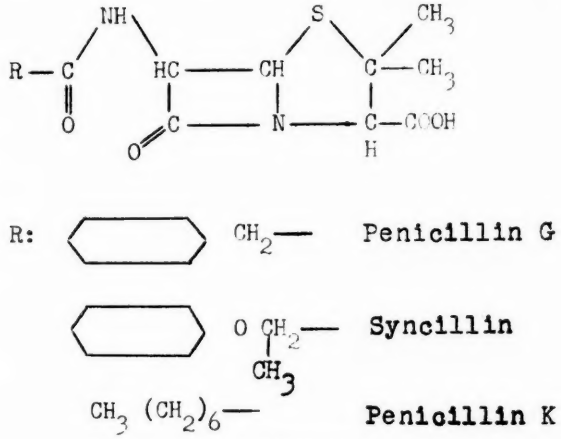


Fig. 1.—Penicillin.

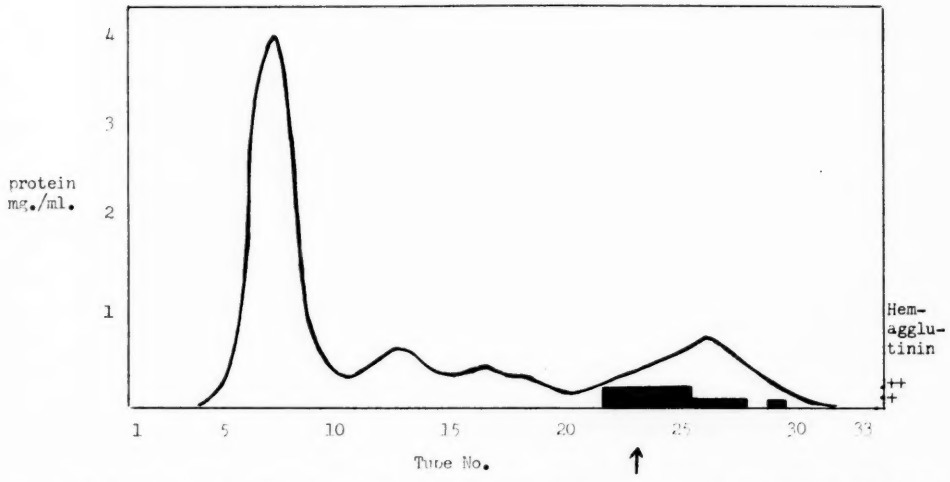


Fig. 2.—Penicillin hemagglutinin. Starch block electrophoresis of serum (Knittle). Serum was applied at the arrow (↑) and the anode was at the left. Barbital buffer was used at pH 8.6, 0.075 M. Half-inch blocks were cut out, eluted with 0.15 M NaCl into separate tubes, and tested individually for protein content (—) and hemagglutinating activity (■).

Zone electrophoresis in potato starch at pH 8.6 has revealed the hemagglutinin to migrate as a fast γ-globulin on two occasions. One of these was with the serum Knittle (Table IV and Fig. 2). The other was with a serum which hemagglutinated but failed to give a Coombs reaction.

TABLE V. INHIBITION OF AGGLUTINATION OF PENICILLIN-COATED CELLS

| PER TUBE | ANTI-PENICILLIN SERUM (WALKER*) | | | | |
|---|---|---|---|---|---|
| | UNDIL. | 1:2 | 1:4 | 1:8 | 1:16 |
| Penicillin | | | | | |
| 100 u. | − | − | − | − | − |
| 50 u. | ± | − | − | − | − |
| 20 u. | + | + | + | ± | − |
| 2 u. | ++ | ++ | ++ | + | − |
| 0 u. | ++ | ++ | ++ | + | − |
| Syncillin | | | | | |
| 125 u. | | − | − | − | − |
| 60 u. | | + | − | − | − |
| 30 u. | | + | − | − | − |
| 15 u. | | + | ± | − | − |
| 0 u. | | ++ | ++ | + | − |

*A 74-year-old man who developed a desquamating rash from procaine penicillin G given for a pneumonitis developing during therapy for congestive heart failure. Recovery was uneventful.

## DISCUSSION

The present small experience in studying penicillin reactions has emphasized how limited is the skin test response alone as a diagnostic tool in the identification of allergy. Lack of positive skin reactions in patients clinically hypersensitive to pollens has led more to attempts at variations in skin testing than to trying basically different methods of assessment of serum antibody or of cellular hypersensitivity. The infrequency of positive skin reactions in drug hypersensitivity has prompted some to the belief that most drug reactions have no immunologic basis, and impetus to this has been provided by the finding of a genetically-determined enzyme defect in primaquine sensitivity. In the current state of activity in the employment of hemagglutination reactions for the assessment of "desensitization," it may be worth some thought that these may have even greater usefulness in diagnosis. Gordon's[4] finding (also Arbesman[5]) of positive ragweed hemagglutination reactions in patients who have had no prior treatment supports this possibility.

In most of the individuals reported in the present study who had experienced penicillin hypersensitivity reactions, it was possible to implicate a hypersensitive state either by positive skin tests or by positive hemagglutination reactions. Of thirteen individuals with a diagnosis of probable penicillin reaction who were tested by *both* criteria, only one was negative by both. Nevertheless, in more extensive testing, more cases certainly will continue to come to light, in which the currently employed criteria for hypersensitivity will demonstrate nothing, in spite of the clinical likelihood that a penicillin reaction has occurred. The development of still newer techniques for identifying the hypersensitive state should be sought for such situations. Just as the penicillin hemagglutination reaction has enhanced our present ability to confirm penicillin allergy in individuals with negative skin tests, so still newer testing methods may in the future be expected to improve further our diagnostic abilities.

As far as the character of penicillin antibody itself is concerned, our experience would indicate that it has the same diversity as has been found for

antibody to blood group antigens. Individual patients vary from one another in the type of immune response they make. Some have elaborated a non-complement-fixing antibody. Others have elaborated antibody that does fix complement. Sometimes direct hemagglutination occurred and the subsequent "Coombs" test was negative. Fudenberg[6] has recently noted that penicillin hemagglutinin is found to be a 19S γ-globulin in some patients and a 7S in others. Neither Fudenberg nor ourselves, however, have observed hemagglutination attributable to a "pre-albumin," as described by Watson.[7] Some patients have developed no detectable serum antibody, but have shown delayed skin reactivity. The specificity of such delayed reactions has not yet been established by the use of penicillinase or synthetic penicillin, but such specificity has been reported for the immediate wheal and flare response. The numbers of patients are as yet too small to indicate a predominate clinical picture corresponding to one or another of these types of immune response.

There is presently no particular reason to emphasize one type of penicillin antibody over another in respect to pathogenetic potential. Indeed current literature emphasizes the occurrence of the hemagglutination reaction with the sera of individuals suffering no apparent hypersensitivity reaction to the drug. Also patients with positive skin tests have been found who subsequently have tolerated penicillin therapy without mishap.[9] Nevertheless, the incidence of positive hemagglutination reactions and of positive skin tests is definitely higher among those who have had penicillin reactions. The higher incidence of positive hemagglutinations among those having reactions has also been found by Heggie.[8] There are quantitative as well as qualitative immunologic factors that are important in determining whether a given antibody response or other change in the immunologic state may permit hypersensitivity symptoms. In addition, there are undoubtedly non-immunologic determinants, presently poorly understood or not appreciated at all, which need description. Possibly some consideration should be given in this respect to the biochemical pathways involved in the substrate tissues and to corresponding alterations in the "intermediates" in the antigen-antibody interaction. The role of previously established disease in modifying the substrate response also needs attention, and more experience among patients with the collagen-vascular group of diseases would be of particular interest.

The specificity of the hemagglutinating antibody for penicillin is confirmed by (a) the ability of small amounts of penicillin G to inhibit the serum reaction, (b) the inability of penicillinase-treated penicillin to coat cells effectively for hemagglutination with known positive sera, and (c) the ability of synthetic penicillin (Penicillin-152) also to inhibit the reaction. This last observation is of additional significance in its implications concerning the use of synthetic penicillin as a substitute antibiotic in patients clinically sensitive to ordinary penicillin preparations. Although some cases may be found in which the synthetic preparation can be used without difficulty, there is no assurance of the safety of this; and the risk must be considered significant.

SUMMARY

Immunologic evidence of penicillin hypersensitivity can frequently be found in patients experiencing penicillin reactions. There is no good correlation between positive skin tests and positive hemagglutinations, each occurring independently of the other. There exist instances of penicillin hypersensitivity based on immune mechanisms other than skin sensitizing antibody, a fact that emphasizes the limitations of the use of skin tests alone for diagnosing allergic conditions. The hemagglutination technique has a high degree of specificity for the penicillin molecule.

ADDENDUM

Recent reports of Levine, B. B. (J. Exper. Med. 112: 1131, 1960) and of de Week, A. L., and Eisen, H. N. (J. Exper. Med. 112: 1227, 1960) indicate that a penicillin metabolite, rather than penicillin itself, may be the active constituent.

REFERENCES

1. Ley, A. B., Harris, J. P., Brinkley, M., Liles, B., Jack, J. A., and Cahan, A.: Circulating Antibody Directed Against Penicillin, Science **127**: 1118, 1958.
2. Vaughan, J. H.: Immunologic Features of Erythrocyte Sensitization. I. Acquired Hemolytic Disease, J. Hematol. **11**: 1085, 1956.
3. Vaughan, J. H., and Waller, M. V.: Immunologic Features of Erythrocyte Sensitization. II. The Nature of Blood Group Antibodies, J. Hematol. **12**: 29, 1957.
4. Gordon, J., Rose, B., and Sehon, A. H.: Detection of "Non-Precipitation" Antibodies in Sera of Individuals Allergic to Ragweed Pollen by an In Vitro Method, J. Exper. Med. **108**: 37, 1958.
5. Arbesman, C. E., Rose, N. R., and Kantor, S. Z.: Immunologic Studies of Ragweed-Sensitive Patients, J. ALLERGY **31**: 333, 1960.
6. Fudenberg, H. H., and German, J. L.: Certain Physical Characteristics of Penicillin Antibody, Blood **15**: 683, 1960.
7. Watson, K. C., Joubert, S. M., and Bennett, M. A. E.: The Occurrence of Hemagglutinating Antibody to Penicillin, Immunology **3**: 1, 1960.
8. Heggie, A. D.: Incidence of Circulating Antibody to Penicillin in Penicillin Hypersensitivity Reactions, New England J. Med. **262**: 1160, 1960.
9. Peck, S. M., Siegal, S., Glick, A. W., and Kurtin, A.: Clinical Problems in Penicillin Sensitivity, J. A. M. A. **133**: 631, 1948.
10. Pillsbury, D. M., Steiger, H. P., and Gibson, F. E.: Management of Urticaria Due to Penicillin, J. A. M. A. **133**: 1255, 1947.

# A COMPARISON OF GRAVIMETRIC AND VOLUMETRIC POLLEN SAMPLERS

*Richard G. Cornell, Ph.D.,* Sarah F. Welch, and Lawrence B. Hall, M.S., Atlanta and Savannah, Ga.*

THE PURPOSE of this paper is to investigate the distributions of pollen clump counts obtained by two types of samplers: gravimetric and volumetric. The gravimetric sampler[1] consists of a collection slide mounted between two horizontal plates. As its name implies it depends on natural processes to capture clumps of pollen. The volumetric sampler[2] employs a black membrane filter in a suitable holder. A metered volume of air is drawn through the filter by a vacuum pump and a flow regulating device. Gravimetric samplers have been in common use for many years. For instance, physicians often use them to measure the prevalence of pollen. However, a volumetric sampler would appear, because of its air volume component, to be a more satisfactory sampler. Despite adverse aerodynamic factors of the device which interfere with its accuracy in the quantitation of the total particulate load of a unit volume of air, it probably is the nearest mechanical proximation of the human nose and it may, therefore, more accurately represent the quantity of particles inhaled than do devices more aerodynamically perfect.

In 1956, the Biophysics Section in the Technical Development Laboratories of the Communicable Disease Center collected pollen from pairs of samplers, one gravimetric and one volumetric, mounted within six feet of each other at varying distances from a pollen source. After two hours of exposure the gravimetric slides were removed from the samplers and stained; then the *Ambrosia elatior* grains were counted within twenty-four hours. After three cubic yards of air were drawn through the volumetric sampler membrane filters, in approximately the same time period as the exposure of the gravimetric slides, they were carefully removed from the holders and were mounted between 2 by 2 inch photographic slide cover glasses. The mounted filters were stored on edge until counting could be done during the winter season. At that time, the filters, in their glass mounts, were illuminated by oblique light by means of which counting could easily be done without staining. A total of 172 pairs of pollen clump counts were obtained during two-hour periods.

From the Statistics Section, Epidemiology Branch, and the Biophysics Section, Technology Branch, of the Communicable Disease Center, Public Health Service, U. S. Department of Health, Education and Welfare, Atlanta, Ga., and Savannah, Ga., respectively.

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*Present address: Department of Statistics, The Florida State University, Tallahassee, Fla.

We shall first examine the correlation between the gravimetric and volumetric counts. Then to better characterize and contrast these two types of samplers we shall investigate separately the distributions of pollen clumps collected by them.

RESULTS

In order to examine the association between counts of pollen clumps obtained by the two sampling methods, the logarithms of the pollen clump counts plus one for the gravimetric samplers are plotted against the corresponding logarithms for the volumetric samplers in Fig. 1. Logarithms are used because the distributions of the logarithms of numbers of clumps plus one are more

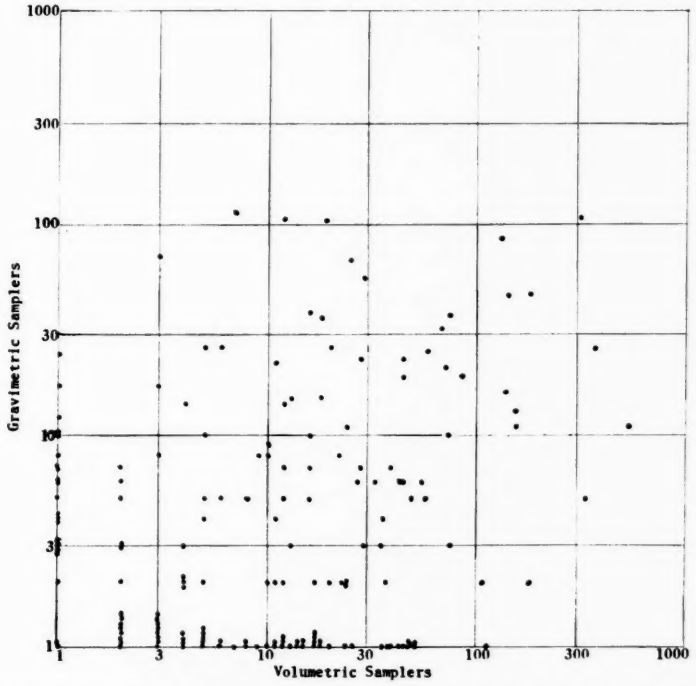


Fig. 1.—A comparison of logarithms of pollen clump counts, plus 1, per two hours for pairs of adjacent gravimetric and volumetric samplers.

nearly symmetrical than the distributions of the actual counts. The points in Fig. 1 are widely scattered and there is only a slight tendency for high gravimetric counts to be associated with high volumetric counts. The product moment correlation coefficient for these logarithms is 0.24. This is much closer to zero, which would indicate no correlation, than it is to one, which would indicate complete positive correlation. This is not a high enough level of correlation to permit precise prediction of, say, a volumetric count to correspond with an observed gravimetric count. This confirms the experience of Cocke[3] who predicted volumetric counts from gravimetric counts and found that they deviated by as much as 56 per cent from concurrent volumetric observations.

Next, the logarithms compared in Fig. 1 are plotted separately as the abscissas on logarithmic normal graph paper in Figs. 2 and 3 for the gravimetric and volumetric samplers, respectively. The proportions on the ordinates at the left are the cumulative proportions with which the corresponding numbers of clumps or fewer clumps are observed. The straight lines were computed to represent the data in Figs. 2 and 3 by Bartlett's method.[4] Expected proportions and, subsequently, frequencies of samples with each number of clumps were determined from the straight lines. These expected frequencies are compared with the observed frequencies of clumps for the gravimetric samplers in Table I and for the volumetric samplers in Table II.

TABLE I. A COMPARISON OF OBSERVED GRAVIMETRIC FREQUENCIES OF POLLEN CLUMP COUNTS PER TWO HOURS WITH EXPECTED FREQUENCIES COMPUTED FROM A LOGARITHMIC NORMAL DISTRIBUTION

| NUMBER OF POLLEN CLUMPS PER TWO HOURS | OBSERVED FREQUENCY O | EXPECTED FREQUENCY E |
|---|---|---|
| 0 | 64 | 61.06 |
| 1 | 17 | 23.22 |
| 2 | 10 | 13.76 |
| 3 | 7 | 9.46 |
| 4 | 10 | 6.88 |
| 5 | 8 | 5.16 |
| 6-7 | 12 | 8.60 |
| 8-9 | 6 | 6.02 |
| 10-12 | 5 | 6.02 |
| 13-15 | 5 | 5.16 |
| 16-20 | 5 | 5.16 |
| 21-30 | 9 | 5.16 |
| 31-45 | 5 | 5.16 |
| 46+ | 9 | 11.18 |
| Total | 172 | 172.00 |

$\chi^2 = \Sigma(O-E)^2/E = 11.27$ with 11 degrees of freedom.

TABLE II. A COMPARISON OF OBSERVED VOLUMETRIC FREQUENCIES OF POLLEN CLUMP COUNTS PER TWO HOURS WITH EXPECTED FREQUENCIES COMPUTED FROM A LOGARITHMIC NORMAL DISTRIBUTION

| NUMBER OF POLLEN CLUMPS PER TWO HOURS | OBSERVED FREQUENCY O | EXPECTED FREQUENCY E |
|---|---|---|
| 0 | 21 | 18.06 |
| 1 | 14 | 16.34 |
| 2 | 11 | 12.04 |
| 3 | 9 | 10.32 |
| 4 | 10 | 8.60 |
| 5-6 | 6 | 12.04 |
| 7-8 | 5 | 10.32 |
| 9-10 | 9 | 8.60 |
| 11-12 | 11 | 5.16 |
| 13-15 | 8 | 8.60 |
| 16-18 | 10 | 6.88 |
| 19-21 | 4 | 5.16 |
| 22-25 | 7 | 5.16 |
| 26-30 | 5 | 5.16 |
| 31-40 | 8 | 8.60 |
| 41-50 | 10 | 5.16 |
| 51-75 | 10 | 8.60 |
| 76-125 | 3 | 6.88 |
| 126+ | 11 | 10.32 |
| Total | 172 | 172.00 |

$\chi^2 = \Sigma(O-E)^2/E = 23.12$ with 16 degrees of freedom.

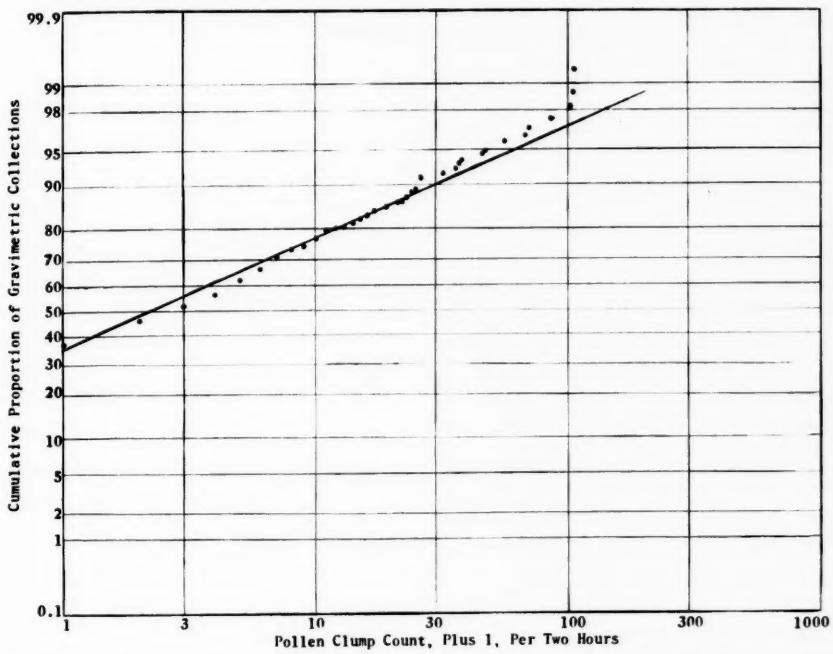


Fig. 2.—Cumulative proportions of gravimetric collections plotted on logarithmic normal graph paper versus pollen clump counts, plus 1, per two hours.

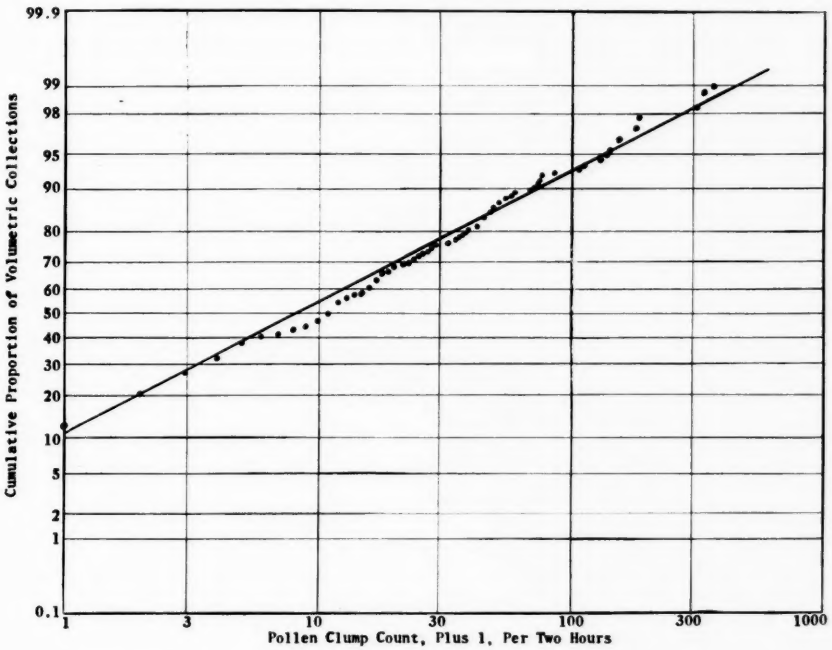


Fig. 3.—Cumulative proportions of volumetric collections plotted on logarithmic normal graph paper versus pollen clump counts, plus 1, per two hours.

The chi-square goodness-of-fit statistic for Table I is 11.27 with 11 degrees of freedom and for Table II is 23.12 with 16 degrees of freedom. The probability, P, of the first chi-square arising when the observations actually come from the fitted distribution is such that $0.25 < P < 0.50$. For the second chi-square value, $0.10 < P < 0.25$.

An attempt was also made to describe the distributions of numbers of clumps for the gravimetric and volumetric samplers by the less skewed negative binomial distribution using the estimation method of maximum likelihood.[5] The calculated mean m and exponent k for the gravimetric counts were 9.419 and 0.2710,

TABLE III. A COMPARISON OF OBSERVED GRAVIMETRIC FREQUENCIES OF POLLEN CLUMP COUNTS PER TWO HOURS WITH EXPECTED FREQUENCIES COMPUTED FROM A NEGATIVE BINOMIAL DISTRIBUTION

| NUMBER OF POLLEN CLUMPS PER TWO HOURS | OBSERVED FREQUENCY O | EXPECTED FREQUENCY E |
|---|---|---|
| 0 | 64 | 65.249 |
| 1 | 17 | 17.187 |
| 2 | 10 | 10.617 |
| 3 | 7 | 7.812 |
| 4 | 10 | 6.210 |
| 5 | 8 | 5.156 |
| 6-7 | 12 | 8.237 |
| 8-9 | 6 | 6.412 |
| 10-12 | 5 | 7.459 |
| 13-15 | 5 | 5.749 |
| 16-20 | 5 | 7.157 |
| 21-30 | 9 | 9.090 |
| 31-45 | 5 | 7.211 |
| 46+ | 9 | 8.454 |
| Total | 172 | 172.00 |

$\chi^2 = \Sigma(O-E)^2/E = 8.045$ with 11 degrees of freedom.

TABLE IV. A COMPARISON OF OBSERVED VOLUMETRIC FREQUENCIES OF POLLEN CLUMP COUNTS PER TWO HOURS WITH EXPECTED FREQUENCIES COMPUTED FROM A NEGATIVE BINOMIAL DISTRIBUTION

| NUMBER OF POLLEN CLUMPS PER TWO HOURS | OBSERVED FREQUENCY O | EXPECTED FREQUENCY E |
|---|---|---|
| 0 | 21 | 27.962 |
| 1 | 14 | 11.622 |
| 2 | 11 | 8.148 |
| 3 | 9 | 6.488 |
| 4 | 10 | 5.475 |
| 5-6 | 6 | 9.034 |
| 7-8 | 5 | 7.380 |
| 9-10 | 9 | 6.282 |
| 11-12 | 11 | 5.484 |
| 13-15 | 8 | 7.118 |
| 16-18 | 10 | 6.115 |
| 19-21 | 4 | 5.349 |
| 22-25 | 7 | 6.205 |
| 26-30 | 5 | 6.602 |
| 31-40 | 8 | 10.450 |
| 41-50 | 10 | 7.901 |
| 51-75 | 10 | 13.099 |
| 76-125 | 3 | 12.384 |
| 126+ | 11 | 8.902 |
| Total | 172 | 172.00 |

$\chi^2 = \Sigma(O-E)^2/E = 29.319$ with 16 degrees of freedom.

respectively, while for the volumetric counts the corresponding estimates were 31.01 and 0.4213. Tables III and IV compare the observed frequencies with the expected negative binomial frequencies for the two types of samplers. For the gravimetric data, chi-square equals 8.045 with 11 degrees of freedom and $0.50 < P < 0.75$; for the volumetric data chi-square equals 29.319 with 16 degrees of freedom and $0.010 < P < 0.025$.

Note that both the logarithmic normal and the negative binomial distributions better describe the distribution of gravimetric clump counts than that of volumetric clump counts. However, the gravimetric count distribution is fitted more closely by the negative binomial distribution than by the logarithmic normal while the opposite is true for the volumetric count frequencies.

## DISCUSSION

One way in which the negative binomial distribution may be generated mathematically is to assume that the probability of capture in different samplers or in the same sampler at different times varies in accordance with a gamma distribution, a flexible unimodal distribution, and that the distribution of clumps for a given sampler and time is Poisson. The latter assumption implies that each pollen clump is sampled randomly and independently. Since the distribution of pollen clump counts for the gravimetric samplers is described very well by the negative binomial distribution, it is reasonable to assume that each gravimetric sampler collects a random sample of the pollen clumps passing over it.

On the other hand, the volumetric samplers placed adjacent to the gravimetric samplers yielded relatively fewer samples with no pollen clumps and the range of the number of clumps captured by the volumetric samplers was much greater. This greater skewness of the pollen clump distribution for the volumetric samplers was borne out by the better fit of the logarithmic normal distribution than the negative binomial distribution to the volumetric data.

## SUMMARY AND CONCLUSIONS

The correlation between pollen clump counts obtained by the two types of samplers is not sufficiently high to predict the performance of one type of sampler from results obtained with the other. The gravimetric sampler would seem to be preferable if the number of pollen clumps which would be expected to fall on a surface without special attraction were desired. However, the volumetric sampler is more appropriate for determining the number of clumps which are injected into the nose because of similarity of construction. The volumetric samplers captured at least one pollen clump more often than did the gravimetric samplers and, in general, the volumetric samplers tended to give higher pollen clump counts even though the two types of samplers were placed side by side.

REFERENCES

1. Durham, O. C., Black, J. H., Glaser, J., and Walzer, M.: Preliminary Report of the National Pollen Survey Committee of the American Academy of Allergy on Proposed Standardization of Pollen Counting Techniques, J. ALLERGY **17**: 178, 1946.
2. Wolf, Harold W., et al.: Sampling Microbiological Aerosols, Public Health Monograph No. 60, U. S. Department of Health, Education and Welfare, Washington, D. C., 1959.
3. Cocke, E. C.: Calculating the Pollen Concentration of the Air, J. ALLERGY **8**: 601, 1937.
4. Bartlett, M. S.: Fitting a Straight Line When Both Variables Are Subject to Error, Biometrics **5**: 207, 1949.
5. Bliss, C. I., and Fisher, R. A.: Fitting the Negative Binomial Distribution to Biological Data and Note on the Efficient Fitting of the Negative Binomial, Biometrics **9**: 176, 1953.

# MEDIASTINAL AND SUBCUTANEOUS EMPHYSEMA COMPLICATING BRONCHIAL ASTHMA IN A NINE-YEAR-OLD MALE

*Thomas W. Payne, Captain, MC, USA, and Leo J. Geppert, Colonel, MC, USA,*
*Fort Sam Houston, Texas*

THIRTY-ONE cases of mediastinal and subcutaneous emphysema complicating bronchial asthma have been reported since Knott described the first case in 1850. Half of these cases have occurred in children. Only one fatal case has been reported.[1] Mediastinal emphysema is mentioned as a finding in only a few of the case reports reviewed, while subcutaneous emphysema has been a constant finding in all cases.[2] An additional case report is now submitted with review of the literature (Table I).

## CASE REPORT

A 9-year-old Negro male was admitted to Brooke General Hospital on Jan. 25, 1958, because of an acute asthmatic attack. He had been seen the morning of the day of admission in the Pediatric Clinic with moderate asthma and given Adrenalin with relief. He was sent home to continue ephedrine and phenobarbital treatment. Later in the day, however, his symptoms increased in severity with audible wheezing and retraction being noted. He returned to the clinic, was again treated with Adrenalin without response, and was thus hospitalized for more vigorous therapy. Review of the past history revealed that this was the child's first hospitalization, but that he had been asthmatic since the age of five years. By history and skin tests he appeared to be allergic to dust, cat and dog hair, grasses, and molds. His asthma was seasonal, being worse in damp, windy, fall weather. The mother estimated that the child had 20 to 30 mild to moderately severe asthmatic attacks each year, which were usually aborted or treated successfully at home.

*Physical Examination.*—The weight on admission was 57½ pounds and height was 53 inches. Pulse rate was 120 with a blood pressure of 110/80 mm. Hg. The child was in acute respiratory distress with suprasternal and infraclavicular retraction. Questions were answered in a gasping manner, but with clear sensorium. Pulmonary rhonchi were heard bilaterally with accompanying musical râles. Cardiac examination was normal, as was the remainder of the physical examination.

*Laboratory Examination.*—The hemoglobin was 13.2 Gm.; the hematocrit was 41; the leukocyte count was 17,400 with 92 per cent neutrophiles, 7 per cent lymphocytes, and 1 per cent eosinophils. Sickle cell preparation was negative. Urinalysis was normal. *Staphylococcus albus,* coagulase negative, was cultured from the nasopharyngeal swab, and alpha-hemolytic streptococcus was recovered from the throat culture. Admission chest x-ray film, dated Jan. 25, 1958, revealed increased bronchovascular markings along the

From the Pediatric Service, Department of Medicine, Brooke General Hospital, Brooke Army Medical Center, Fort Sam Houston, Texas.
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superior portion of the left heart border with an unusual area of radio density, thought to be segmental atelectasis. Repeat chest roentgenogram, dated Jan. 28, 1958, revealed mediastinal emphysema with migration of air into the subcutaneous tissue of the right neck region (Figs. 1 and 2). Repeat x-ray examination on Feb. 4, 1958, revealed clearing of the emphysematous process. Intermediate strength PPD was reported negative at twenty-four hours and forty-eight hours.

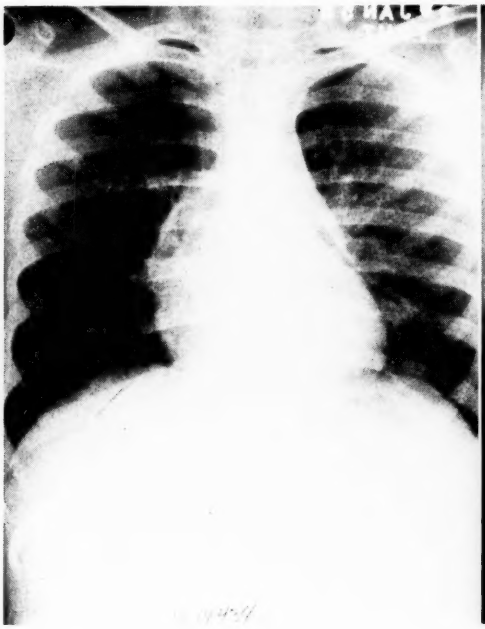

Fig. 1.

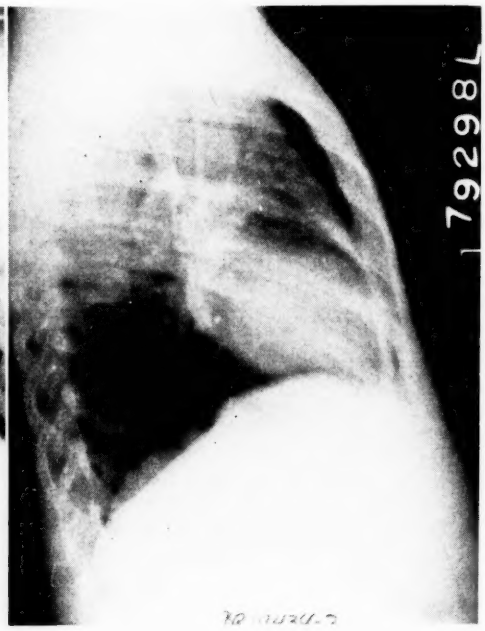


Fig. 2.

Fig. 1.—Demonstration by x-ray of mediastinal emphysema with air adjacent to the left heart border and subcutaneous emphysema of the right supraclavicular area.

Fig. 2.—Lateral roentgenogram which demonstrates anterior mediastinal emphysema.

*Hospital Course.*—The child was placed in an oxygen tent, given intravenous fluid therapy, and sedated. Adrenalin, saturated solution of potassium iodide, ephedrine, and penicillin were given. The child received only minimal relief from this regimen, became more dyspneic and irritable, and complained of substernal chest pain. Because of resistance to twelve hours of the above therapy, ACTH, 10 mg. intramuscularly every six hours was begun, and within six to eight hours the child was much improved. The wheezes and rhonchi gradually disappeared over the next three to four days, but the substernal pain continued without other associated symptoms. On the morning of Jan. 28, 1958, four days after the acute asthmatic attack, subcutaneous emphysema was noted in the right supraclavicular area. At this time a chest roentgenogram revealed mediastinal emphysema as well (Figs. 1 and 2). The child continued to improve, however, and was discharged Feb. 4, 1958, completely recovered both clinically and radiologically.

## COMMENT

The diagnostic features of mediastinal emphysema as a complication of bronchial asthma were first described in 1888 by Mueller, while Hamman in 1937 directed current attention to this entity.[5] It occurs ten times as often in

TABLE I. (FROM SCHWARTZ[2])*

| NO. | AUTHOR | AGE | SEX | DURATION OF ASTHMA | DURATION OF EMPHYSEMA (DAYS) |
|---|---|---|---|---|---|
| 1 | Knott[2] | 5 | F | Unknown | 21 |
| 2 | Watson[2] | 18 | F | Lifelong | 9 |
| 3 | Calverly[2] | 17 | M | 11 years | 4 |
| 4 | Whitby[2] | 25 | M | Lifelong | Unknown |
| 5 | Kahn[2] | 8 | F | 6 years | 10 |
| 6 | MacDermott[2] | 22 | M | 6 years | 5-6 |
| 7 | Scheltema[2] | 30 | M | First attack | 7 |
| 8 | Artagaveytia and Zanzi[2] | 18 | M | Lifelong | 8 |
| 9 | Seremini and Berro[2] | Unk | F | Unknown | Unknown (few) |
| 10 | Mourigan and Cervino[2] | 21 | F | Lifelong | 2 |
| 11 | Kruysveldt[2] | 22 | F | ? Lifelong | 14 |
| 12 | Davidson[2] | 9 | F | 8 years | 7 |
| 13 | Pastorino[2] | 7 | F | Unknown | 6-7 |
| 14 | Pastorino[2] | 14 | M | Lifelong | 10 |
| 15 | Sheldon and Robinson[2] | 16 | M | 1 year | 4 |
| 16 | Kirsner[2] | 38 | M | First attack | 20 |
| 17 | Bridge[2] | 6 | M | 3 years | 6 |
| 18 | Dietrich[2] | 5 | F | 2 years | 6 |
| 19 | Dietrich[2] | 7 | M | 5 years | 7 |
| 20 | Rosenberg and Rosenberg[2] | 3 | F | 2½ years | 12 |
| 21 | Van Fleet, Miller, and Scott[2] | 3 | F | Lifelong | Unknown |
| 22 | Van Fleet et al.[2] | 3 | M | 2 years | 10 |
| 23 | Van Fleet et al.[2] | 28 | M | 7 years | 9 |
| 24 | Van Fleet et al.[2] | 19 | F | 17 years | 13 |
| 25 | Skinner[2] | 10 | Unk | 3 years | 10 |
| 26 | Francis[4] | 31 | M | 12 years | 7 |
| 27 | Francis[4] | 13 | F | Lifelong | 10 |
| 28 | Schwartz[2] | 24 | M | 21 years | 9 |
| 29 | Peterson[1] | 20 | F | 8 years | |
| 30 | Creel[6] | 20 | M | ? Lifelong | 7 |
| 31 | Kahn and Rouse[3] | 5 | M | ? 6 months | 5 |
| 32 | Payne and Geppert | 9 | M | 4 years | 5 |

*Prognosis is evidently good because only Case No. 29 died.

males as in females.[6] Hansel, in *Clinical Allergy*, points out that the aforementioned complication should be suspected when an asthmatic patient complains of substernal chest pain during an attack.[7] In all cases reviewed, subcutaneous emphysema was first noted in the neck and then spread to the face and chest. From these areas, air can then spread to the upper extremities, and, rarely, to the abdomen and thighs. The mechanism of formation of mediastinal and subcutaneous emphysema which seems most acceptable to date is that described in animal experiments performed by Macklin and Macklin in 1943.[2] They demonstrated that the essential factor necessary for pulmonary interstitial emphysema was a pressure gradient between air in the alveolus and in the underlying connective tissue leading to a rupture of the base of the marginal type alveolus.[2] Air then seeps out into the pulmonary interstitial space, compresses the pulmonary vessels, and then travels along the vascular sheaths to the mediastinum. From the mediastinum the air then migrates into the subcutaneous tissue.[2] The extent of circulatory embarrassment depends obviously

on the amount of air pressure exerted upon the heart and great vessels. This embarrassment is relieved as the air migrates from the mediastinum out into the subcutaneous tissue. If air ruptures out into the pleural space from the mediastinum, pneumothorax results. Most investigators today no longer subscribe to the older pleural bleb theory as the source of mediastinal and subcutaneous emphysema.[2] Physical findings consist of substernal pain, cyanosis, dyspnea, crunching noises on auscultation synchronous with cardiac activity, and palpable subcutaneous emphysema. On chest x-ray films, air can be seen in the mediastinum and subcutaneous tissue. Because of the benign nature of this disorder, conservative therapy is the one of choice. With rest and limitation of vigorous activity, complete recovery occurs within four to fifteen days. The single reported death with this disorder was believed to be due to a combination of asphyxia due to asthma and to vascular complications caused by excessive mediastinal pressure on the heart and great vessels.[1]

SUMMARY

An additional case of mediastinal and subcutaneous emphysema complicating bronchial asthma is reported which brings the total number of reported cases to thirty-two. Fifteen of these have occurred in children.

The literature is reviewed and tabulation of reported cases is brought up to date.

The mechanism as to the origin and migration of air in this entity is briefly discussed.

REFERENCES

1. Peterson, H.: Fatal Case of Bronchial Asthma Complicated by Mediastinal and Subcutaneous Emphysema, J. ALLERGY **18:** 413, 1947.
2. Schwartz, E.: Spontaneous Mediastinal and Subcutaneous Emphysema Complicating Bronchial Asthma, J. ALLERGY **16:** 279, 1945.
3. Kahn, I. S., and Rouse, J. W. H.: Interstitial Mediastinal Emphysema Due to Bronchial Asthma, J. ALLERGY **22:** 111, 1951.
4. Francis, N.: Subcutaneous Emphysema During Asthma, Ann. Allergy **2:** 349, 1944.
5. Derbes, V. J., Engelhardt, H. T., and Sodeman, W. A.: Unusual Complications of Bronchial Asthma: Air in Extrapulmonary Space, Ann. Allergy **3:** 21, 1945.
6. Creel, W. F.: Spontaneous Mediastinal Emphysema, Texas State J. Med. **45:** 853, 1949.
7. Hansel, F. K.: Clinical Allergy, St. Louis, 1927, The C. V. Mosby Company, p. 613.

# BRONCHODILATORS: SOME QUALITATIVE AND QUANTITATIVE PHARMACOLOGIC CORRELATES IN THE GUINEA PIG

*K. W. Dungan and P. M. Lish, Evansville, Ind.*

BRONCHODILATOR drugs relax either the normal or spastic tonus of the smooth muscle cells of guinea pig trachea in vitro, and the relative potencies of these drugs are proportional to the potencies found clinically. Many drugs relax the spastic guinea pig trachea in vitro but do not relax the spontaneous tracheal tonus, and such agents generally are not clinically useful bronchodilators. Antihistaminic agents, for example, relax histamine spasms even though these agents are predominantly bronchoconstrictors[1]; and certain drugs (for example, Adiphenine) inhibit barium chloride-induced spasms but these drugs do not relax spontaneous tracheal tonus. Similarly, acetylcholine-induced spasms are antagonized by "non-bronchodilator" anticholinergic agents and serotonin spasms are antagonized by "non-bronchodilator" antiserotonin agents.

The inherent difficulty of preparing tracheal tissue in the form of a chain[2] limits the usefulness of this in vitro test as a routine screening procedure. Patterson[3] authored a modification based on the substitution of a "spiral strip" for the original "chain" preparation. The use of the spiral strip involves no loss in sensitivity of the tissue, and the qualitative response of the tracheal spiral, upon exposure to spasmogenic agents (histamine or barium chloride) or to spasmolytic agents (epinephrine or aminophylline), is equivalent to that of the chain preparation.

Extensive tests with known bronchodilators have been completed using the method of Patterson[3] modified by the terminal attainment of maximal relaxation of each tracheal spiral in each determination. The data illustrate the utility of the tracheal spiral test, as modified, in determining the relative potencies of bronchodilators and for studying the mechanisms of action of bronchodilators. Certain in vivo test results related to the in vitro data have also been included.

## METHODS

*In Vitro Experiments on the Guinea Pig Tracheal Spiral.*—An adult guinea pig, either sex, was killed by a blow on the head. The trachea was immediately removed, placed in bath solution, and extraneous tissue carefully excised. A wooden dowel of appropriate diameter was inserted longitudinally through the tracheal bore, and the tissue cut spirally with a sharp scalpel.

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The spiral strip was suspended vertically in a 10 ml. chamber and its movements recorded kymographically by attachment to an isotonic, gravity writing lever. The chamber solution was aerated with bubbling oxygen and maintained at 37.5° C., pH 7.35. It had the following composition, in grams per liter: NaCl—8.0; KCl—0.2; $CaCl_2$ (anhydrous)—0.2; $MgCl_2 \cdot 6H_2O$—0.1; $NaHCO_3$—0.5; $NaH_2PO_4 \cdot H_2O$—0.044; and dextrose—0.5. The writing lever recorded a twentyfold magnification on a smoked drum moving at 0.08 cm. per minute, and exerted a sustained tension of 2.0 Gm. on the tissue throughout the experiment. Under these conditions, the equilibrium length of the strip was 4.5 to 5.0 cm.

Five in vitro tests were used to evaluate the effects of drugs on the tracheal spiral. Each test shared the following common features: (1) the spiral strip was allowed an equilibrium period, after suspension in the chamber, of at least 60 minutes; (2) all additions to the bath fluid were contained in a total volume of 0.2 ml., and all concentrations in the text are stated per milliliter of bath fluid; (3) test agents remained in contact with the tissue until the maximum effect was observed, or for a maximum interval of 40 minutes; (4) maximal relaxation of each strip was attained by the terminal addition of 10 μg papaverine (SMCP; supra-maximal concentration of papaverine); and (5) each spiral strip was from a separate guinea pig and used for a single trial of one test drug only.

The five in vitro tests used in these studies on the tracheal spiral of the guinea pig are listed below, with procedural comment as necessary:

1. Effect on spontaneous tonus of the normal tracheal spiral.

2. Effect on barium chloride-induced contraction of the normal tracheal spiral; the test agent was added while the strip was in a sustained spasm induced by 200 μg barium chloride. The relatively nonspecific spasmogenic action of the latter agent diminished the probability of obtaining a "false" bronchodilator action, due to a specific drug antagonism, such as occurs with atropine versus a pilocarpine-induced spasm.

3. Effect on spontaneous tonus of the tracheal spiral in the presence of the dichloro analogue of isoproterenol (DCI); 1.0 μg DCI was added to the bath fluid prior to introduction of the tracheal strip.

4. Effect on barium chloride-induced contraction of the tracheal spiral in the presence of DCI; 0.4 μg DCI was added to the bath following the production of a sustained tissue spasm by 200 μg barium chloride. The test agent was added 20 minutes after the addition of DCI.

5. Effect on spontaneous tonus of the tracheal spiral from the guinea pig pretreated with reserpine; 4 mg. of reserpine per kilogram was administered intraperitoneally to the guinea pig on each of the 2 days immediately preceding its use.

*In Vivo Aerosol Studies in the Guinea Pig.*—Asthma-like attacks were induced in guinea pigs by subjecting the animals, in a closed spray-chamber, to aerosols of 1.0 per cent histamine diphosphate. De Vilbiss vaporizers delivered

the histamine solution in particle sizes ranging from 0.3 to 2.0 $\mu$. The guinea pigs were removed from the chamber immediately following signs of dyspnea or coughing, and re-exposed two to four hours later, after treatment with a test agent. Repeated experiments demonstrated that the first exposure to histamine aerosol did not significantly influence the time of onset of dyspnea during the second exposure, in unprotected animals. Thus, each animal served as its own control, and eight to twelve animals were used for each dosage level of a test agent. The time from beginning of exposure to onset of symptoms was termed the "pre-dyspneic interval." The effectiveness of a test agent in extending the pre-dyspneic interval was determined at its time of peak effect after subcutaneous administration.

*Isolated Atria of Guinea Pigs.*—Reserpine, 4 mg. per kilogram intraperitoneally, was administered to guinea pigs on each of the two days immediately preceding their use.

Atria from freshly killed pigs were freed from ventricular mass and fat tissue and suspended in a 50 ml. bath containing oxygenated Chenoweth's solution[4] (pH 7.4) maintained at a temperature of 37.5° C. The contractile force and rate of the beating atria were recorded on a Sanborn recorder using a Statham strain gauge. The atria were suspended in the bath so that the left atrium was proximal to the strain gauge. A tension of 1.0 Gm. was maintained on the atria throughout the experiment.

Introduction of the atria into the bath medium was followed by a one hour stabilization period. A decrease from the initial rate and an increase from the initial contractile force were usually observed during this period. After stabilization, aqueous solutions of test drugs were added to the bath at a constant volume of 0.5 ml.

The reserpine-treated guinea pigs used in these tests were also used for the in vitro "reserpine-tracheal spiral" studies mentioned above.

*Test Drugs.*—The agents studied for bronchodilator and/or cardiac actions were: one xanthine derivative–theophylline ethylenediamine (aminophylline); six phenethanolamines–ephedrine HCl, isoxsuprine HCl, $\beta$-hydroxy-N-isopropyl-3,4-dichlorophenethylamine HCl (DCI), isoprophenamine HCl, dl-isopropylarterenol HCl (isoproterenol), l-epinephrine bitartrate; and one benzylisoquinoline–papaverine HCl. Barium chloride, histamine diphosphate, and reserpine were also used. All of the drugs used in the in vitro studies were prepared daily in aqueous solution, except reserpine, which was administered in vivo as a 2 mg. per milliliter solution in 5 per cent ascorbic acid solution, pH 5.0. In the in vivo aerosol studies, test drugs were administered as solutions in 0.2N phosphate buffer, pH 5.5. Epinephrine concentrations are expressed in terms of the base; all other agents in terms of salts.

*Statistical Treatment.*—Regression lines were constructed of the log dose—response relationship for each drug used in each of the test procedures. The standard deviation from regression, the regression coefficient with its standard error, and "Student's t" were derived according to Snedecor.[5]

TABLE I. THE COMPARISON OF TWO METHODS OF MEASUREMENT OF THE EFFECT OF DRUGS ON THE SPONTANEOUS TONUS OF THE ISOLATED TRACHEAL SPIRAL OF THE GUINEA PIG

| | DECREASE IN TRACHEAL TONUS (MM.) | | | | DECREASE IN TRACHEAL TONUS (% OF MAXIMUM) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | MAXIMUM EFFECT | | POTENCY RATIO | | | MAXIMUM EFFECT | | POTENCY RATIO | |
| TEST AGENT | $ED_{20\ mm.}$* | MEAN DECREASE (MM.) | MAXIMUM CONCN. STUDIED (μg/ML.) | $ED_{20\ mm.}$ | $ED_{12.5\%}$* | $ED_{75\%}$* | MEAN DECREASE (MM.) | MAXIMUM CONCN. STUDIED (μg/ML.) | $ED_{12.5\%}$ | $ED_{75\%}$ |
| Aminophylline | 24.0 ± 4.5 | 49.1 | 80.0 | 1.0 | 26.0 ± 2.2 | 59.0 ± 5.5 | 84.7 | 80.0 | 1.0 | 1.0 |
| Ephedrine | 0.54 ± 0.02 | 19.0 – 24.0 | 1.0 – 64.0 | 44.0 | 0.84 ± 0.28 | † | 45.0 – 52.0 | 1.0 – 64.0 | 31.0 | — |
| Papaverine | 0.28 ± 0.075 | 51.0 | 2.0 | 86.0 | 0.33 ± 0.035 | 1.0 ± 0.11 | 94.0 | 2.0 | 79.0 | 59.0 |
| Isoxsuprine | 0.11 ± 0.047 | 35.0 | 0.8 | 218.0 | 0.072 ± 0.017 | 0.29 ± 0.07 | 95.0 | 0.8 | 361.0 | 204.0 |
| DCI | 0.09 ± 0.04 | 24.0 – 30.0 | 0.2 – 51.2 | 267.0 | 0.06 ± 0.016 | † | 59.0 – 63.0 | 0.2 – 51.2 | 433.0 | — |
| Isoprophenamine | 0.021 ± 0.0045 | 29.0 | 0.0312 – 0.0624 | 1,143.0 | 0.0165 ± 0.0025 | † | 71.0 | 0.0312 – 0.0624 | 1,576.0 | — |
| Epinephrine | 0.0087 ± 0.0024 | 53.1 | 0.04 | 2,759.0 | 0.0078 ± 0.0015 | 0.027 ± 0.005 | 83.0 | 0.04 | 3,333.0 | 2,185.0 |
| Isoproterenol | 0.0006 ± 0.0015 | 38.0 | 0.008 | 40,000.0 | 0.00084 ± 0.00016 | 0.0041 ± 0.0008 | 85.0 | 0.008 | 30,952.0 | 14,390.0 |

*Concentration, in μg/ml. of bath fluid (± standard error), causing the stipulated millimeter and/or per cent decrease in tonus; values interpolated graphically.

†Not attainable.

A standard error from regression was calculated by dividing the standard deviation by the square root of the number of responses about a single dose. The same number of responses was obtained with each test dose within a given experiment, the number varying from 4 to 7 in the in vitro experiments and from 8 to 12 in the in vivo tests. The standard error for an interpolated dose was read from the graph as ± the mean horizontal distance of the standard error lines from the regression line.

Statistical significance was achieved whenever $P \leq 0.05$.

## RESULTS

*In Vitro Experiments.*—

1. Drug effect on spontaneous tonus of the normal tracheal spiral: Table I shows the results obtained in studies of eight drugs in vitro. The results are given in terms of (1) the decrease in tonus, expressed in millimeters, and (2) the percentage of the maximum relaxation obtainable, where maximum relaxation is defined as that effect produced by 10 $\mu$g papaverine (SMCP). The concentration of drug producing a 20 mm. decrease in tonus ($ED_{20\ mm.}$) was selected as the basis of comparison. This approaches the maximal relaxation obtained with certain of the test agents studied, i.e., ephedrine and DCI. The mean of the maximal decrease in tonus produced by the addition of SMCP in 181 trials with the eight test agents was 47 ± 1.3 mm. (standard error). Thus, the response corresponding to a 20 mm. decrease in tonus occurred at 42.5 per cent of the maximal decrease in tonus. The respective doses of each of the test drugs required to produce this effect are shown in Table I.

The $ED_{20\ mm.}$ concentration did not vary significantly from the $ED_{42.5\%}$ concentration with any of the agents tested. However, the mean variation about the $ED_{42.5\%}$ values was only one third as great as that associated with the $ED_{20\ mm.}$ values. Regression coefficients derived using the percentage method were greater in every instance (significantly so with all agents other than ephedrine) than the corresponding values derived using the millimeter decrease in tonus method. The latter slopes, obtained with both isoproterenol and isoprophenamine, did not differ significantly from the horizontal, whereas their respective percentage slopes were highly significant. Fig. 1 illustrates the differences in variation and slope obtained by the two methods, using isoproterenol as the test agent.

The relaxing effect of certain drugs (ephedrine, DCI, and isoprophenamine) plateaued at 50 to 70 per cent of the maximum relaxation produced by SMCP, and increased concentration afforded no greater effect. The data plotted in Fig. 2 contrast this action of ephedrine with the activity of the much more potent sympathomimetic, epinephrine.

2. Drug effect on barium chloride-induced contraction of the normal tracheal spiral: Pertinent results obtained with six test agents are entered in Table II. Each of these drugs, except ephedrine, completely abolished the barium-induced

contraction. With DCI, however, this was accomplished only at very large concentrations, while lesser doses caused 15 to 35 per cent reduction. These results observed for DCI, ephedrine, and isoxsuprine are compared in Fig. 3.

With the exception of DCI, the test drugs maintained the same order of potency relative to aminophylline as was observed in the spontaneous tonus test, and pronounced differences in the potency of an individual drug were not observed when the two test procedures were compared (Table II).

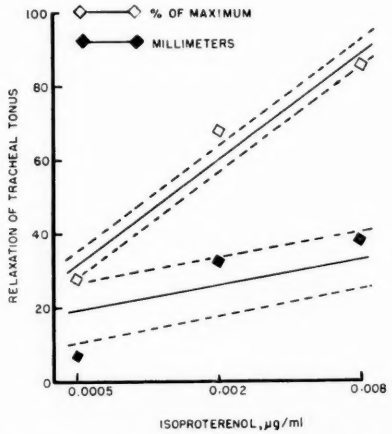


Fig. 1.—Comparison of two methods of determining the smooth muscle relaxant effect of isoproterenol on the tracheal spiral of the guinea pig in vitro. Broken lines are ± 1.0 standard error from the solid regression lines.

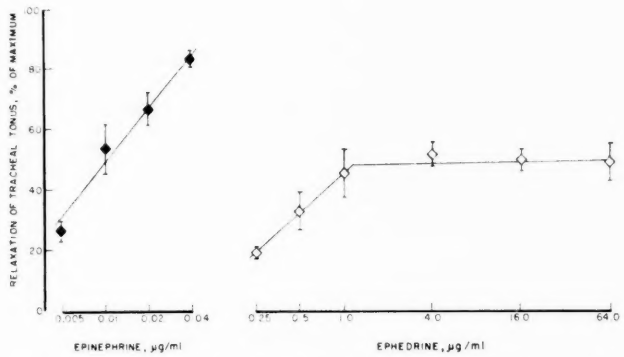


Fig. 2.—Comparison of the effect of epinephrine and ephedrine on the spontaneous tonus of the tracheal spiral of the guinea pig in vitro. Vertical bars represent ± 1.0 standard error of the mean. Note inability of ephedrine to relax tracheal tonus by more than 50 per cent of maximum.

3. Effect of DCI on the action of bronchodilators on the tonus of the normal tracheal spiral: The presence of DCI profoundly decreased the ability of both isoxsuprine and isoproterenol to relax the spontaneous tonus of the tracheal spiral. DCI did not, however, significantly affect the activity of either aminophylline or papaverine. Fig. 4, A shows records obtained with the test agents before and after the addition of DCI.

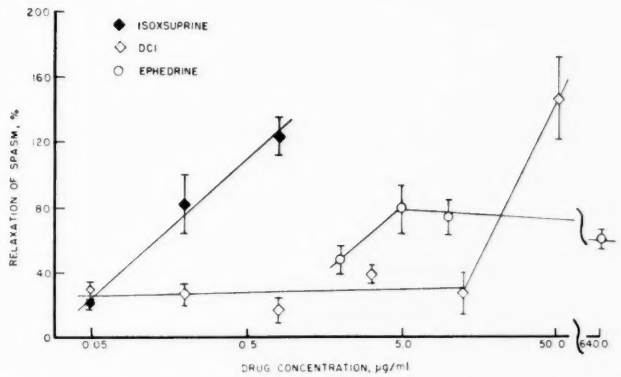


Fig. 3.—Comparison of the effect of three phenethanolamines on barium chloride-induced spasm of the isolated tracheal spiral of the guinea pig. Vertical bars represent ± 1.0 standard error of the mean. Relaxation > 100 per cent indicates reduction below the pre-spasm base line.

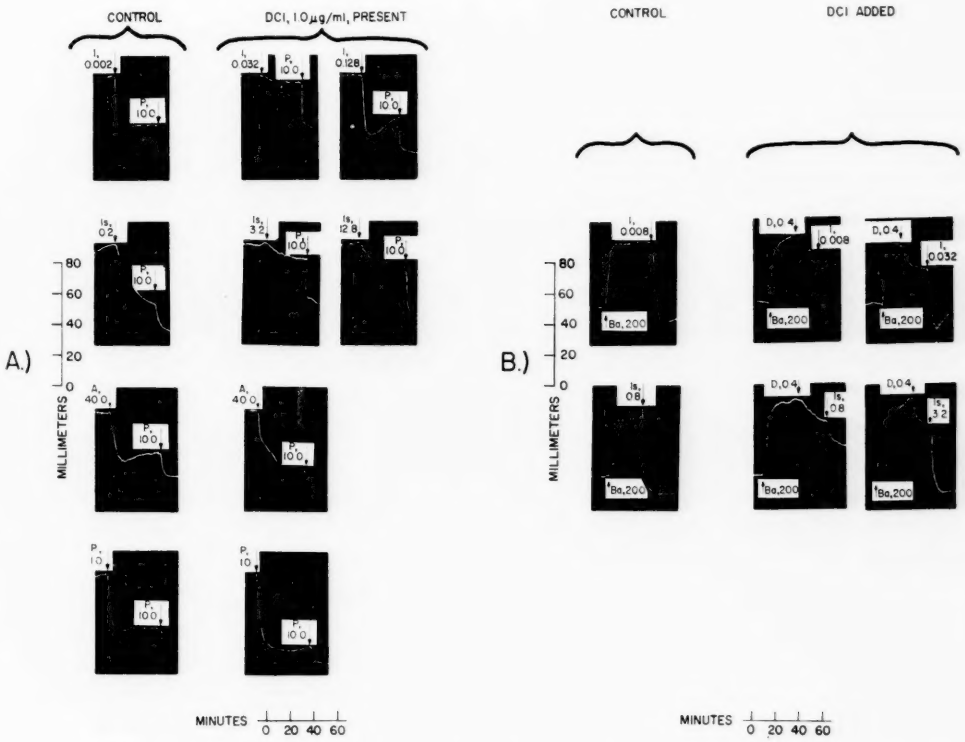


Fig. 4.—Isolated tracheal spiral of the guinea pig. All concentrations are µg/ml.

*A*, DCI antagonism of the relaxing action of isoproterenol (*I*) and isoxsuprine (*Is*), but not of aminophylline (*A*) or papaverine (*P*), on spontaneous tonus.

*B*, DCI (*D*) antagonism of the relaxing action of *I* and *Is* on barium chloride (*Ba*)-induced spasm.

TABLE II. EFFECT OF DRUGS ON BARIUM CHLORIDE-INDUCED CONTRACTION OF THE TRACHEAL SPIRAL OF THE GUINEA PIG IN VITRO

| TEST AGENT | $ED_{75}$%* | MAXIMUM EFFECT | | ACTIVITY RATIO: EFFECT ON SPONTANEOUS TONUS‡ / EFFECT ON $BaCl_2$-INDUCED SPASM§ |
|---|---|---|---|---|
| | | MEAN† DECREASE (%) | MAXIMUM CONCN. STUDIED (μg/ML.) | |
| Aminophylline | 34.5 | 110.0 | 50.0 | 1.7 |
| Ephedrine | 4.3 | 80.0 | 5-640.0 | 0.5 |
| Papaverine | 0.7 | 100.0 | 1.0 | 1.4 |
| Isoxsuprine | 0.2 | 125.0 | 0.8 | 1.5 |
| DCI | 22.0 | 143.0 | 51.2 | 0.004 |
| Isoproterenol | 0.0023 | 141.0 | 0.008 | 1.8 |

*Concentration (μg/ml.) decreasing spasm by 75 per cent.

†Values > 100 per cent indicate reduction below the resting base line.

‡Concentration causing 75 per cent (42.5 per cent for ephedrine and DCI) of maximum relaxation.

§Concentration returning spasm 75 per cent (42.5 per cent for ephedrine and DCI) toward base line.

The isoproterenol concentration required to relax the tracheal tonus by 75 per cent of maximum was approximately fifty times greater in the presence of DCI than on the normal spiral. Isoxsuprine did not produce 75 per cent of maximum relaxation, in the presence of DCI, even at concentrations 175 times as large as the dose producing this effect on the normal spiral (Table III).

TABLE III. EFFECT OF DCI ON THE RESPONSE OF THE ISOLATED TRACHEAL SPIRAL OF THE GUINEA PIG TO OTHER DRUGS

| IN VITRO TEST CONDITION | TEST AGENT | | | | ACTIVITY RATIO: $ED_{75}$% IN PRESENCE OF DCI / $ED_{75}$% IN ABSENCE OF DCI |
|---|---|---|---|---|---|
| | NAME | CONCN. (μg/ML.) | MEAN* EFFECT | $ED_{75}$% † (μg/ML.) | |
| Spontaneous tonus in presence of DCI (1.0 μg/ml.) | Isoxsuprine | 3.2 | 30.6 ± 6.1‡ | > 51.2 | > 177.0 |
| | | 12.8 | 33.2 ± 2.9 | | |
| | | 51.2 | 29.8 ± 15.0 | | |
| | Isoproterenol | 0.032 | 29.2 ± 5.1 | | |
| | | 0.128 | 68.0 ± 3.5 | 0.21 | 51.2 |
| | | 0.512 | 94.2 ± 1.7 | | |
| | Aminophylline | 40.0 | 66.3 ± 2.9 | — | 1.0§ |
| | Papaverine | 1.0 | 81.6 ± 2.5 | — | 1.0§ |
| $BaCl_2$-induced spasm; DCI (0.4 μg/ml.) added prior to test agent | Isoxsuprine | 0.2 | 40.7 ± 4.7 | 0.73 | 3.7 |
| | | 0.8 | 75.0 ± 4.8 | | |
| | | 3.2 | 99.8 ± 11.7 | | |
| | Isoproterenol | 0.002 | 33.2 ± 4.6 | 0.0066 | 2.9 |
| | | 0.008 | 57.4 ± 8.5 | | |
| | | 0.032 | 161.6 ± 16.7 | | |

*Spontaneous tonus test—measured as per cent of maximum relaxation obtainable. $BaCl_2$ spasm test—measured as per cent reduction in induced contraction.

†Concentration causing 75 per cent of maximum relaxation.

‡Standard error.

§Compared at one concentration only.

4. Effect of DCI on the antispasmodic action of bronchodilators: The antagonism of both isoxsuprine and isoproterenol toward barium chloride-induced spasms of the tracheal spiral was reduced by the prior exposure of the spiral

to DCI. After DCI, three- to four-fold increase in dosage was required with either isoxsuprine or isoproterenol to obtain relaxation of the spasm comparable to that produced in the spiral not exposed to DCI (Table III). The DCI effect could be completely overcome, however, by large doses of either isoxsuprine or isoproterenol (Fig. 4, *B*).

5. Effect of reserpine on the action of bronchodilators on the trachea and heart: The effects of isoxsuprine and ephedrine on the tonus of tracheae derived from guinea pigs pretreated with reserpine are compared with controls in Table IV. Reserpine pretreatment in vivo did not significantly alter the ability of either isoxsuprine or ephedrine to relax spontaneous tracheal tonus in vitro. All animals used in this test exhibited systemic signs characteristic of reserpine treatment (depression, ptosis, and diarrhea) at the time they were sacrificed.

TABLE IV. THE EFFECT OF RESERPINE TREATMENT IN VIVO ON THE REACTIVITY OF THE TRACHEAL SPIRAL OF THE GUINEA PIG IN VITRO TO ISOXSUPRINE AND EPHEDRINE

| TEST AGENT | | CONTROLS | | RESERPINE-TREATED | | DIFFERENCE BETWEEN MEANS (P VALUE) |
|---|---|---|---|---|---|---|
| NAME | CONCN. (μg/ML.) | NUMBER TRIALS | MEAN EFFECT* | NUMBER TRIALS | MEAN EFFECT* | |
| Isoxsuprine | 0.2 | 5 | 75.0 ± 7.3† | 5 | 81.6 ± 1.2 | 0.3 |
| Ephedrine | 0.5 | 5 | 33.3 ± 6.1 | 5 | 40.6 ± 2.5 | 0.3 |

*Relaxation of spontaneous tonus expressed as per cent of the maximum relaxation obtainable.

†Standard error.

Previous studies in this laboratory have established that reserpine pretreatment of rabbits rendered the isolated atria less responsive to the positive inotropic and chronotropic action of ephedrine while not affecting, significantly, the action of isoxsuprine or isoproterenol. The effect of reserpine pretreatment on the isolated atria from the guinea pigs used in the present in vitro bronchodilator tests is shown in Table V. Pretreatment of the guinea pigs with reserpine in vivo decreased the response of the isolated atria to ephedrine, but did not significantly affect the response of the atria to isoxsuprine or isoproterenol.

TABLE V. THE EFFECT OF RESERPINE TREATMENT IN VIVO ON THE RESPONSE OF THE ISOLATED ATRIA OF THE GUINEA PIG TO ISOXSUPRINE, EPHEDRINE, AND ISOPROTERENOL

| TEST AGENT | | CONTROLS MEAN INCREASE (%) | | RESERPINE-TREATED MEAN INCREASE (%) | | DIFFERENCE BETWEEN MEANS (P VALUE) | |
|---|---|---|---|---|---|---|---|
| NAME | CONCN. (μg/ML.) | AURICULAR RATE | AURICULAR CONTRACTILE FORCE | AURICULAR RATE | AURICULAR CONTRACTILE FORCE | RATE | FORCE |
| Isoxsuprine | 0.5 | 45.7 ± 5.7* | 28.9 ± 6.5 | 41.8 ± 3.2 | 18.6 ± 7.2 | >0.5 | >0.2 |
| Ephedrine | 4.0 | 56.5 ± 3.7 | 51.8 ± 8.1 | 22.8 ± 4.8 | 15.7 ± 5.3 | <0.005 | <0.025 |
| Isoproterenol | 0.005 | 85.8 ± 12.7 | 130.4 ± 18.4 | 96.4 ± 16.2 | 96.5 ± 8.6 | >0.05 | >0.1 |

*Standard error.

*In Vivo Aerosol Studies in the Guinea Pig.—*

Distinct differences occurred among the test agents relative to their respective abilities to extend the time required to produce symptoms of bronchoconstriction with aerosolized histamine. These differences are illustrated graphically in Fig. 5. Both isoproterenol and aminophylline increased the pre-dyspneic

interval by periods in excess of four minutes, isoproterenol being from 4,000 to 8,000 times as potent as aminophylline. The maximum increase obtained with ephedrine or isoprophenamine was about two minutes, and the maximum obtained with DCI was about 0.5 minute. When compared at the level of doses causing 75 seconds increase in the symptom onset interval, ephedrine and isoprophenamine exhibited approximately the same activity and were about three times more potent than aminophylline.

The inability of DCI, ephedrine, and isoprophenamine to extend the predyspnea interval by more than about one half of that observed with either isoproterenol or aminophylline paralleled the in vitro tracheal spiral results. As shown in Table I, the maximum relaxation of the isolated tracheal spiral tonus produced by DCI, ephedrine, or isoprophenamine was less than 75 per cent of the maximum relaxation obtainable, whereas this effect was exceeded by both isoproterenol and aminophylline.

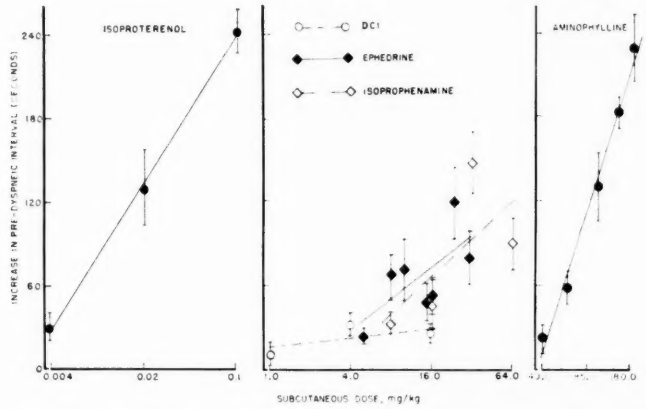


Fig. 5.—The effect of drugs in vivo in antagonizing bronchoconstriction induced by histamine-aerosol in the guinea pig. "Pre-dyspneic interval" represents the time from exposure to onset of dyspnea. Each point represents the mean of 8-12 paired observations. Vertical bars indicate ± 1.0 standard error of the mean.

## DISCUSSION

Papaverine was chosen as the logical agent to produce maximum relaxation of the guinea pig tracheal spiral. It is the classic prototype of drugs having general depressant activity on all types of smooth muscle and this effect is cited by Goodman and Gilman[6] as being due to a direct action on the muscle cell, unrelated to innervation. Adequate doses of such an agent should, therefore, produce the maximum muscle relaxation obtainable, irrespective of either the existing intrinsic muscle tone (whether spontaneous or drug-affected) or the anatomical or physiological disposition of its nerve supply. (Hawkins and Paton[7] have elicited strong evidence for the occurrence of both cholinergic and adrenergic ganglia in the guinea pig trachea.) In the tests on the spontaneous tonus of the normal spiral, the mean relaxation produced by 10 $\mu$g papaverine, when added after one of the test agents, did not vary significantly among the

eight drugs tested. Thus, the prior and continued exposure of the tracheal muscle to a xanthine derivative, sympathomimetics (both weak and strong), or papaverine itself did not significantly alter the response of the muscle to the 10 μg dose of papaverine; neither did the existence of a sustained muscle spasm, induced by 200 μg barium chloride, significantly affect the relaxation ultimately produced by 10 μg papaverine. Once the 10 μg papaverine effect was attained, there was no further tracheal relaxation with the subsequent introduction of (1) 20 μg doses of the sympathomimetics, epinephrine or isoproterenol, respectively, (2) 20 μg doses of papaverine, (3) 200 μg doses of the xanthine derivative, aminophylline, or (4) 400 μg doses of the antihistaminic, promethazine hydrochloride. (Hawkins[1] stated that this latter dose of the antihistamine produced a maximal relaxation of the guinea pig tracheal chain.) These findings, therefore, afford ample evidence that the relaxation produced by 10 μg papaverine was, in fact, maximal relaxation.

Expression of drug effect in terms of the maximum obtainable effect offers certain advantages. Less variation occurs about a mean drug effect, expressed in terms of per cent of the maximum effect, than when the results are expressed in terms of an absolute measurement. Variations associated with differences in technique, which occur among different laboratories, are also avoided since percentage estimates of drug effect are relatively unaffected, for example, by differences in lever magnifications and tensions applied to the tissue. The most important advantage, however, is the clear illustration of pronounced differences among drugs, even among agents belonging to the same pharmacological "class," relative to their maximum quantitative effects.

The correlation of results obtained by the in vivo aerosol studies with those from the isolated tracheal spiral tests agrees, in general, with the findings of other investigators[8] using the tracheal chain preparation. This parallelism between in vivo and in vitro findings has been shown in these studies to include the maximum quantitative effects possible with the various agents tested. The present results are also in accord with clinical impressions that the extent of bronchodilation obtained with aminophylline or isoproterenol is greater than that possible with ephedrine.[9]

DCI has been shown by Powell and Slater[10] to block epinephrine and isoproterenol inhibition of pilocarpine-induced spasms of the isolated guinea pig tracheal chain. Similar results were obtained in the present studies employing the tracheal spiral. The inhibitory action of either isoproterenol or isoxsuprine on spontaneous tonus or barium chloride-induced spasm was effectively antagonized by DCI. The DCI block was overcome by larger doses of either of the inhibitory agents, however. This latter finding is in agreement with the competitive nature of the DCI antagonism of isoproterenol as found by Powell and Slater[10] and by Moran and Perkins[11] when studying cardiac contractile force in anesthetized open-chest dogs. The failure of DCI to inhibit papaverine activity, as illustrated herein with the tracheal spiral in vitro, was demonstrated in vivo by Lish and associates[12] using a uterine preparation in the anesthetized dog.

Several investigators[13-15] have reported that the vasoconstrictor and pressor effects of ephedrine and certain other phenylalkylamines are reversibly antagonized, in various species, by the prior treatment of the animal with reserpine. This antagonism has been attributed by Burn and Rand[13] to the dispersal of norepinephrine from the effector organ by reserpine. In the present experiments, pretreatment with reserpine in the guinea pig did not alter the inhibitory action of either ephedrine or isoxsuprine on the isolated tracheal spiral. However, the positive inotropic and chronotropic effects of ephedrine on the isolated atria from the same reserpine-treated pigs were significantly decreased when compared to the effects observed on atria from nontreated pigs. These results suggest a possible difference in the mechanisms subserving the cardiac stimulant and tracheal muscle relaxant actions, respectively, of ephedrine. However, they may also reflect a differential susceptibility between the two tissues toward the effect of the parenterally administered reserpine. More definite conclusions must await a quantitative measurement of the effect of reserpine on the norepinephrine content of tracheal muscle.

SUMMARY

1. A series of bronchodilator agents was examined employing the guinea pig tracheal spiral in vitro. Drug effect was expressed as a per cent of the maximal relaxation obtained by the terminal introduction of a supramaximal concentration of papaverine.

2. Distinct differences were observed among the drugs tested, relative to their maximum quantitative effects as tracheal muscle relaxants in vitro. These differences were well correlated with the results of in vivo histamine aerosol studies in the guinea pig and, also, with clinical impressions of the relative potencies of these drugs.

3. DCI markedly antagonized the relaxing effect of both isoproterenol and isoxsuprine on either spontaneous tonus or barium-induced spasm of the isolated tracheal spiral. The DCI block could be overcome by higher concentrations of either relaxant. The activities of aminophylline and papaverine, respectively, were not decreased in the presence of DCI.

4. In vivo treatment of guinea pigs with parenteral reserpine did not decrease ephedrine activity on the subsequently isolated tracheal spiral. However, ephedrine-induced effects on the isolated atria from the same reserpine-treated animals were significantly less than control effects on nontreated animals.

REFERENCES

1. Hawkins, D. F.: Bronchoconstrictor and Bronchodilator Actions of Antihistamine Drugs, Brit. J. Pharmacol. **10:** 230, 1955.
2. Castillo, J. C., and de Beer, E. J.: The Tracheal Chain: A Preparation for the Study of Antispasmodics With Particular Reference to Bronchodilator Drugs, J. Pharmacol. & Exper. Therap. **90:** 104, 1947.
3. Patterson, R.: The Tracheal Strip: Observations on the Response of Tracheal Muscle, J. ALLERGY **29:** 165, 1958.
4. Chenoweth, M. B., and Kolle, E. S.: An Isolated Heart Perfusion System Adapted to the Determination of Nongaseous Metabolites, J. Lab. & Clin. Med. **31:** 600, 1946.

5. Snedecor, G. W.: Statistical Methods, ed. 5, Ames, Iowa, 1956, The Iowa State College Press, p. 125.
6. Goodman, L. S., and Gilman, A.: The Pharmacological Basis of Therapeutics, ed. 2, New York, 1955, The Macmillan Co., p. 251.
7. Hawkins, D. F., and Paton, W. D. M.: Responses of Isolated Bronchial Muscle to Ganglionically Active Drugs, J. Physiol. **144**: 193, 1958.
8. Parker, J. M., McColl, J. D., and Ferguson, J. K. W.: A Comparison of the Bronchodilator Activity of Some 1- and 7-Substituted Xanthines in the Guinea Pig, J. Pharmacol. & Exper. Therap. **118**: 359, 1956.
9. Herxheimer, H.: Die Symptomatische Behandlung des Bronchiolasthmas mit Medikamenten, Allergie Asthma **2**: 235, 1956.
10. Powell, C. E., and Slater, I. H.: Blocking of Inhibitory Adrenergic Receptors by a Dichloro Analogue of Isoproterenol, J. Pharmacol. & Exper. Therap. **122**: 480, 1958.
11. Moran, N. C., and Perkins, M. E.: Adrenergic Blockade of the Mammalian Heart by a Dichloro Analogue of Isoproterenol, J. Pharmacol. & Exper. Therap. **124**: 223, 1958.
12. Lish, P. M., Hillyard, I. W., and Dungan, K. W.: The Uterine Relaxant Properties of Isoxsuprine, J. Pharmacol. & Exper. Therap. **129**: 438, 1960.
13. Burn, J. H., and Rand, M. J.: Effect of Reserpine on Vasoconstriction Caused by Sympathomimetic Amines, Lancet **1**: 673, 1958.
14. Burn, J. H., and Rand, M. J.: The Action of Sympathomimetic Amines in Animals Treated With Reserpine, J. Physiol. **144**: 314, 1958.
15. Maxwell, R. A., Povalski, H., and Plummer, A. J.: A Differential Effect of Reserpine on Pressor Amine Activity and Its Relationship to Other Agents Producing This Effect, J. Pharmacol. & Exper. Therap. **125**: 178, 1959.

# A COMPARISON OF THE IMMUNOLOGIC AND PHYSIOLOGIC RESPONSES OF NORMAL AND ALLERGIC INDIVIDUALS

*Sidney Leskowitz, Ph.D., and Francis C. Lowell, M.D., Boston, Mass.*
*With the technical assistance of Lucio B. Phillipino, B.S., and Ann Z. Schapiro, B.S.*

IN ANY CONSIDERATION of the causes of atopic allergy in a restricted portion of the population at large, at least two reasonable hypotheses deserve consideration. These are: (1) allergy develops because of a qualitatively or quantitatively altered ability to respond immunologically to the antigens (such as, pollen, danders) with which they come in contact, and (2) people become allergic by virtue of a qualitatively or quantitatively altered reactivity to the pharmacologic intermediates released by the reaction of antigens and antibodies in tissues of mucosal surfaces or in the blood stream.

The present work was undertaken in an attempt to determine whether the immunologic response to two well-defined polysaccharide antigens differed significantly in allergic and otherwise normal individuals in terms of both amounts of precipitating antibody produced and appearance of positive skin reactivity following immunization. At the same time, both groups were skin tested with several of the pharmacologic intermediates implicated in allergic reactions to see if the two groups differed in skin reactivity to these agents.

## MATERIALS AND METHODS

*Selection of Groups.*—Patients were selected from the allergy clinic for this study on the basis of the following criteria: (1) allergic rhinitis present for two or more years with one or more clear-cut seasonal exacerbations per year, (2) definitely positive skin reactions to two or more of the following pollen extracts: birch, oak, plantain, timothy, and ragweed, and (3) age between 18 and 60 years.

Normal subjects were selected in whom no history of atopic disease could be elicited. Negative skin tests to the above-mentioned pollen extracts were not a criterion, although, as it turned out, most chanced to be negative.

From the Department of Bacteriology and Immunology, Harvard Medical School and the Department of Medicine, Massachusetts General Hospital, Boston, Mass.
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*Antigens.*—Type VII pneumococcal polysaccharide* (SVII) and dextran N-279† were chosen for the quantitative estimate of antibody production. SVII is known to be a good antigen in man,[1] and its use as an antigen for estimation of immune response in man has been reported previously.[2, 3] Dextran N-279 produces a small but still measurable response in man[4] and might thus select more readily the exceptional antibody producer. Antibodies to both antigens are easily measured by quantitative precipitin test and the antigens are free of measurable protein contaminants.

*Immunization.*—All subjects were given 80 μg SVII subcutaneously and 1 mg. of dextran N-279 intradermally in two separate sites. Three weeks later they were bled, the sera harvested, preserved with merthiolate (1:10,000) and phenol (0.25 per cent), and stored at 3° C.

*Antibody Analyses.*—Antibody analyses were done with slight modifications as described by Heidelberger and his co-workers.[1] To two 3 ml. aliquots each of the pre- and post-injection bleedings were added 10 μg of dextran and 30 μg of SVII, respectively. A third aliquot had nothing added and was used as a serum blank. After incubation for one hour at 37° C., the tubes were stored for one week (with daily resuspension of precipitates) in the refrigerator. The precipitates were then spun and washed with saline in the cold and analyzed using the Folin-Ciocalteau reagent. A color value for the reagent used was determined with a purified sample of human γ-globulin.‡ If large amounts of precipitate were formed (in excess of 100 μg N), an additional portion of antigen was added to the supernatant serum.

In the initial analyses, after removal of the precipitate resulting from addition of the first antigen, the supernatant solutions were reacted with the second antigen, and antibody to it was measured. Thus, in one aliquot of a serum the antibody to dextran was precipitated and measured first and then the antibody to SVII; and, in another aliquot, the reverse procedure was followed. Since both procedures gave identical measurements (within experimental error), demonstrating lack of cross reactions between the two antigens, successive absorption was abandoned in favor of simultaneous assay of both antibodies in separate aliquots in order to conserve time.

*Skin Tests.*—Three separate sets of skin tests were performed on all subjects in this study.

1. Before and after immunization, all subjects were skin tested by the intracutaneous injection of 0.01 to 0.02 ml. of phenolized saline solutions of SVII (80 μg per milliliter), dextran 279 (1 mg. per milliliter), and the saline alone. Reactions were traced on cellophane sheets at 10 minutes and the wheal and erythema recorded in millimeters.

*Supplied by Lederle Laboratories, Pearl River, N. Y.
†Supplied by Dr. Paul Maurer, Seton Hall Medical School, N. J.
‡Supplied by Professor E. A. Kabat, College of Physicians and Surgeons, N. Y.

TABLE I. RESULTS OF SCRATCH TESTS WITH COMMON ALLERGENS ON ALLERGIC AND CONTROL SUBJECTS

| PATIENT | RAGWEED 1:40 | BIRCH 1:40 | H. DUST 1:10 | OAK 1:40 | DOG 1:10 | TIMOTHY 1:40 |
|---|---|---|---|---|---|---|
| | | | *Skin Reactions in Allergic Patients* | | | |
| 1 | 0 | 0 | +++ | 0 | 0 | 0 |
| 2 | ++++ | ++++ | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | ++++ |
| 4 | 0 | 0 | ++ | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | ++++ | +++ | +++ | 0 | 0 | 0 |
| 7 | ++++ | +++ | + | +++ | 0 | ++++ |
| 8 | + | + | 0 | +++ | 0 | ++++ |
| 9 | ++++ | 0 | +++ | 0 | 0 | +++ |
| 10 | ++++ | ++++ | ++++ | ++++ | +++ | 0 |
| 11 | ++++ | ++++ | 0 | ++++ | 0 | ++++ |
| 12 | +++ | 0 | ++ | 0 | 0 | ++ |
| 13 | ++++ | ++++ | 0 | ++++ | 0 | +++ |
| 14 | ++++ | 0 | ++ | 0 | + | +++ |
| 15 | ++++ | + | + | ++ | 0 | +++ |
| 16 | 0 | 0 | ++ | 0 | 0 | 0 |
| 17 | +++ | ++ | + | + | 0 | 0 |
| 18 | ++++ | 0 | 0 | 0 | 0 | 0 |
| 19 | ++++ | +++ | 0 | 0 | ++ | +++ |
| 21 | ++++ | + | 0 | 0 | 0 | + |
| 22 | ++++ | ++ | 0 | + | 0 | ++++ |
| 23 | +++ | ++ | ++ | +++ | 0 | 0 |
| 24 | +++ | ++++ | 0 | +++ | 0 | +++ |
| 25 | 0 | 0 | ++ | 0 | 0 | + |
| 27 | ++++ | 0 | 0 | 0 | 0 | 0 |
| | | | *Skin Reactions in Nonallergic Controls* | | | |
| CONTROL | | | | | | |
| 1 | ++++ | ++++ | +++ | ++++ | + | ++++ |
| 2 | 0 | 0 | ++ | 0 | 0 | 0 |
| 3 | + | 0 | ± | ± | ± | 0 |
| 4 | 0 | 0 | ± | ± | ± | 0 |
| 5 | 0 | 0 | ± | ± | ± | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | ± | 0 | 0 | 0 |
| 9 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 | 0 | +++ |
| 11 | +++ | 0 | 0 | +++ | 0 | +++ |
| 12 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 0 | 0 | + | 0 | 0 | 0 |
| 16 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | + | 0 | ++ | 0 | ++ | + |
| 18 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21 | 0 | 0 | 0 | 0 | 0 | 0 |
| 22 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | 0 | 0 | ++ | 0 | ++ | + |
| 24 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 0 | +++ | +++ | ++ | ++ | +++ |
| 27 | 0 | 0 | ± | 0 | ± | 0 |
| 28 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | 0 | 0 | 0 | 0 | 0 | 0 |

2. All subjects were scratch tested with glycerinated extracts of ragweed, birch, oak, and timothy pollens (1-40), house dust (1-40), and dog dander (1-10). Reactions were recorded as in Table I.

3. All subjects were skin tested with intracutaneous injections of 0.01 to 0.02 ml. of saline solutions of serotonin (100 $\mu$g per milliliter), histamine (10 $\mu$g and 1 $\mu$g per milliliter), and the histamine releaser Compound 48/80* (10 $\mu$g and 1 $\mu$g per milliliter). Reactions were recorded as in Table I.

For simplicity of recording in tables, all skin test results were scored on the following basis:

$-$ = $<$ 4 mm. edema and no erythema.
$+$ = 4-8 mm. edema and up to 10 mm. erythema.
$++$ = 4-8 mm. edema and 10-20 mm. erythema.
$+++$ = 8 mm. edema and $>$ 20 mm. erythema.
$++++$ = $>$ 8 mm. edema and $>$ 25 mm. erythema.

*Operational Procedure.—*

A. On the subject's first visit, the following steps were carried out in the order indicated. (1) A pre-immunization blood specimen was drawn; (2) skin tests with SVII and dextran were performed; (3) if not previously performed in the Allergy Clinic, scratch tests with the six allergens were done; and (4) immunizing injections of SVII and dextran were given.

B. On the subject's second visit 3 weeks later (1) a post-immunization blood specimen was drawn; (2) skin tests with SVII and dextran were performed; and (3) skin tests with serotonin, histamine, and Compound 48/80 were performed.

## RESULTS

Table I lists the results of scratch tests with the 6 common allergens on all individuals in this study. As expected, skin reactivity of the allergic group to these allergens was pronounced; whereas, only the occasional nonallergic individual (1, 11, 26) showed reactivity. Although allergic subject 5 showed no positive scratch tests, repeat intradermal tests were markedly positive. It is of some interest that control subject 1, who showed such marked skin reactions despite absence of clinical symptoms, developed typical allergic rhinitis during June and July for the first time the year after this study. Control subject 26 was an English visitor living in this country for just one year. His future allergic course would be of interest.

The results of skin tests with pharmacologic reagents are shown in barographs in Fig. 1. All subjects gave positive reactions to the test dose of serotonin used, the average reaction for each group being about a 3 plus. Both test doses of histamine and the histamine releaser, Compound 48/80, gave entirely comparable reactions in the two groups of individuals.

*Supplied by Burroughs-Wellcome & Co., Inc., Tuckahoe, N. Y.

Table II lists the results of the immunologic studies with dextran and SVII as antigens.

TABLE II. IMMUNE RESPONSE TO DEXTRAN N-279 AND TYPE VII PNEUMOCOCCAL POLYSACCHARIDE

| | RESPONSE TO DEXTRAN | | | | RESPONSE TO SVII | | | |
|---|---|---|---|---|---|---|---|---|
| | μg AbN/3 ML. | | SKIN TEST | | μg AbN/3 ML. | | SKIN TEST | |
| PATIENT | PRE | POST | PRE | POST | PRE | POST | PRE | POST |
| C- 1 | 1 | 5 | – | + | 23 | 124 | ++ | +++ |
| C- 2 | 1 | 23 | + | ++++ | 12 | 315 | +++ | ++++ |
| C- 3 | 6 | 12 | + | ++++ | 28 | 51 | – | ++ |
| C- 4 | 6 | 13 | – | + | 8 | 80 | – | + |
| C- 5 | 6 | 20 | + | ++++ | 35 | 58 | – | + |
| C- 6 | 1 | 11 | – | + | 11 | 188 | – | ++++ |
| C- 7 | 0 | 0 | + | + | 5 | 47 | – | +++ |
| C- 8 | 0 | 5 | – | + | 8 | 61 | – | + |
| C- 9 | 6 | 5 | – | – | 14 | 33 | + | + |
| C-10 | 4 | 10 | – | – | 12 | 160 | – | +++ |
| C-11 | 0 | 0 | – | – | 10 | 37 | + | + |
| C-12 | 0 | 1 | – | – | 11 | 92 | – | +++ |
| C-13 | 5 | 12 | – | + | 23 | 50 | +++ | ++ |
| C-14 | 0 | 1 | – | + | 47 | 107 | – | ++ |
| C-16 | 7 | 8 | – | +++ | 24 | 56 | – | ++ |
| C-17 | 1 | 2 | ++ | + | 14 | 31 | ++ | +++ |
| C-18 | 11 | 38 | + | +++ | 61 | 116 | +++ | +++ |
| C-19 | 7 | 8 | – | + | 73 | 61 | +++ | +++ |
| C-20 | 4 | 7 | – | + | 24 | 75 | – | +++ |
| C-21 | 4 | 0 | – | – | 6 | 79 | – | +++ |
| C-22 | 5 | 6 | + | + | 12 | 128 | + | ++++ |
| C-23 | 12 | 13 | ++ | +++ | 21 | 33 | ++ | +++ |
| C-24 | 5 | 13 | ++ | + | 31 | 89 | +++ | +++ |
| C-25 | 4 | 9 | – | + | 8 | 111 | – | +++ |
| C-26 | 0 | 2 | – | – | 1 | 31 | – | +++ |
| C-27 | 6 | 8 | – | – | 30 | 75 | – | ++ |
| C-28 | 10 | 17 | + | + | 25 | 33 | + | + |
| C-29 | 6 | 12 | – | + | 44 | 103 | +++ | +++ |
| C-30 | 4 | 8 | – | +++ | 29 | 73 | – | ++ |
| (29) average | 4.2 | 9.6 | | | 22.4 | 86.1 | | |
| A- 1 | 1 | 2 | – | + | 6 | 76 | – | +++ |
| A- 2 | 1 | 0 | + | + | 28 | 39 | – | +++ |
| A- 3 | 5 | 14 | – | +++ | 36 | 102 | – | +++ |
| A- 4 | 5 | 8 | – | – | 9 | 43 | – | – |
| A- 5 | 3 | 17 | – | ++ | 6 | 34 | – | ++ |
| A- 6 | 1 | 20 | – | +++ | 132 | 240 | ++ | +++ |
| A- 7 | 3 | 9 | – | + | 25 | 50 | +++ | +++ |
| A- 8 | 4 | 10 | – | + | 45 | 53 | ++ | +++ |
| A- 9 | 1 | 4 | – | + | 30 | 62 | – | +++ |
| A-10 | 1 | 11 | – | +++ | 20 | 70 | – | +++ |
| A-11 | 3 | 15 | – | + | 50 | 216 | – | + |
| A-12 | 1 | 2 | – | + | 0 | 100 | – | +++ |
| A-13 | 0 | 4 | – | + | 9 | 96 | +++ | +++ |
| A-14 | 2 | 5 | + | +++ | 32 | 279 | +++ | ++++ |
| A-15 | 2 | 14 | – | + | 7 | 94 | – | ++++ |
| A-16 | 2 | 5 | – | + | 67 | 117 | – | +++ |
| A-17 | 4 | 11 | – | ++ | 25 | 279 | + | +++ |
| A-18 | 1 | 5 | + | – | 13 | 83 | +++ | +++ |
| A-19 | 1 | 3 | + | + | 4 | 13 | + | ++ |
| A-21 | 0 | 5 | – | + | 12 | 76 | +++ | +++ |
| A-22 | 0 | 5 | – | – | 36 | 105 | +++ | ++ |
| A-23 | 4 | 5 | + | + | 41 | 85 | + | +++ |
| A-24 | 7 | 11 | – | + | 34 | 48 | ++ | + |
| A-25 | 3 | 3 | – | + | 44 | 70 | – | +++ |
| A-27 | 4 | 7 | – | + | 32 | 88 | + | + |
| (25) average | 2.2 | 8.1 | | | 31 | 100.7 | | |

Of the twenty-nine control subjects, five had pre-immunization levels of antibody for dextran greater than 6 μg N per 3 ml., and only one of these gave a skin reaction greater than 1 plus. Two other individuals with levels below this also gave skin reactions greater than 1 plus. Of the allergic subjects, only one had an antibody level greater than 6 μg N per 3 ml., and none gave skin reactions greater than 1 plus. The post-immunization levels of eighteen out of twenty-nine control subjects (62 per cent) and twelve out of twenty-five (48 per cent) allergic subjects rose above 6 μg AbN per 3 ml. Seven

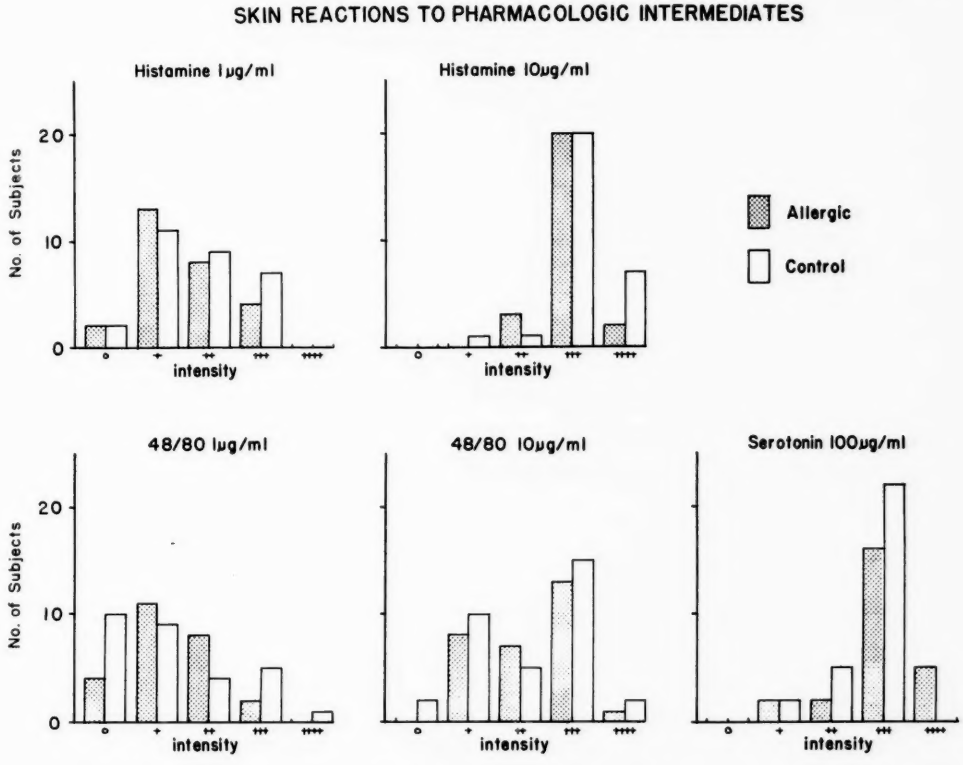


Fig. 1.—Skin reactions to histamine, serotonin, and Compound 48/80.

of these eighteen controls (39 per cent) and five of these twelve allergic subjects (42 per cent) developed definitely positive skin reactions (more marked than 1 plus). Thus, a significant antibody response to dextran occurred in approximately 50 per cent of all individuals studied, with comparable development of positive skin reactivity in both groups.

Much higher levels of antibody to SVII were present in all individuals prior to immunization, perhaps attributable to greater contact with pneumococcus type VII or cross-reacting antigens throughout life. Seven controls (24 per cent) and twelve atopic individuals (48 per cent) had levels above 30 μg AbN per 3 ml. Whether this difference is attributable to the somewhat greater age (hence longer exposure) of the allergic individuals or to their

atopic status is not determinable by the present study. The concomitant presence of skin reactivity of 2 plus or greater was found in four (57 per cent) of these controls and five (42 per cent) of the atopic individuals having antibody levels over 30 μg N per 3 ml. It should be noted that several individuals of each group had skin reaction of 2 plus or more, despite antibody levels below 30 μg N per 3 ml.

Virtually all individuals in this study showed considerable increases in antibody levels in response to immunization with SVII. Average titers rose from 22 to 86 μg AbN per 3 ml. in control subjects and from 31 to 100 μg AbN per 3 ml. in allergic subjects. In most, but by no means all, individuals, this was attended by a similar increase in skin reactions to SVII, so that only six (25 per cent) of the control and four (16 per cent) of the allergic subjects remained unreactive following immunization. It could not be determined with certainty whether the unreactivity of several allergic subjects (A-11, A-27) with very high levels on antibody to SVII was attributable to medication with antihistamines for control of their allergies, despite instructions to refrain from their use the day of skin testing.

## DISCUSSION

Atopic individuals differ from otherwise normal individuals in at least one important observable respect, namely, that they manifest a marked degree of skin reactivity to common air-borne allergens. Almost certainly this cannot be attributed to differences in degree of skin reactivity to the pharmacologic intermediates tested, or to differences in the amount of histamine releasable in the skin as estimated from response to Compound 48/80. The results in Fig. 1 confirm previous reports[7, 8] and do not immediately suggest any reasonable working hypothesis based on physiologic reactivity in the skin to explain the development of allergy. However, the possibility still remains that differences in physiologic reactivity in certain organs exist.

A more reasonable and more widely entertained hypothesis to account for allergy is the development by such individuals of either quantitative or qualitative differences in antibody response to relatively innocuous air-borne antigens. There is still considerable discussion[5] as to whether this ability to respond is hereditary and/or whether there may be a defect not in the immunologic apparatus but, perhaps, in some prior stage, such as, excessive permeability of mucosa to antigens.

Kabat[6] has reviewed the appearance of skin reactivity and allergic reactions to pneumococcal polysaccharides and, in particular, to dextran. These antigens were commended to allergists[6] for additional study on the basis of their known abilities to elicit such reactivity and their known immunologic specificity and chemical structure. The present study using such antigens has revealed no quantitative differences (as determined by measurement of precipitable antibody) or qualitative differences (as determined by the appearance of skin reactivity) in the immunologic response made by atopic or normal individuals.

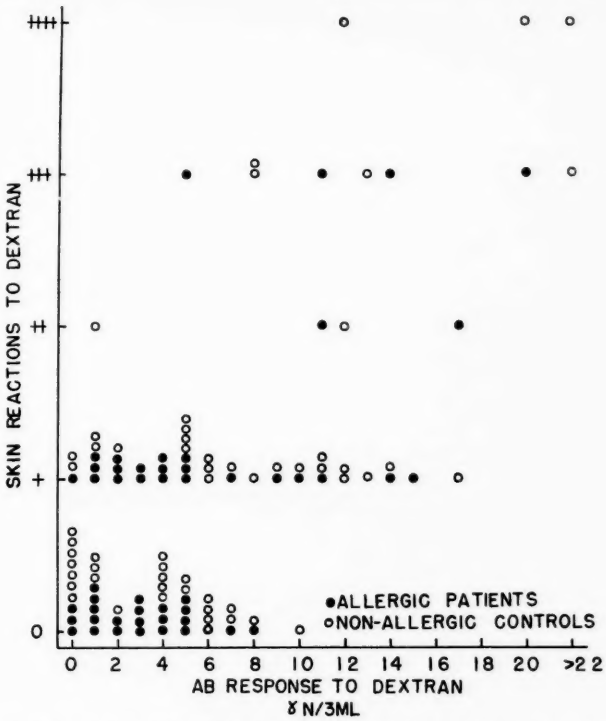


Fig. 2.—Relation of skin reactivity and levels of circulating antibody to dextran in all subjects.

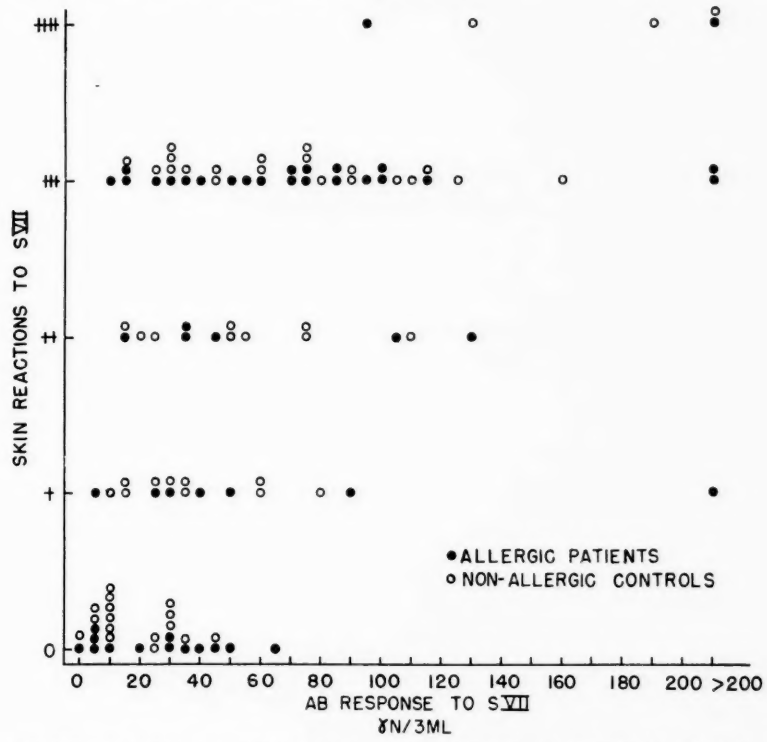


Fig. 3.—Relation of skin reactivity and levels of circulating antibody to SVII in all subjects.

However, an apparent relation was seen between the amount of precipitating antibody found and the skin reaction in any particular individual, regardless of group. Thus, in Fig. 2, when skin reactions to dextran are plotted against antibody levels for all individuals, a distribution roughly characteristic of biologic response patterns results. A similar situation is seen in Fig. 3 with the results obtained for SVII. It may be noted that under the conditions of the experiment the dextran and SVII systems do not appear to be of equal effectiveness on a quantitative basis for producing skin reactivity; very much larger levels of antibody to SVII were seen with less skin reactivity than was the case with the dextran. This difference, however, is more apparent than real, since considerably different amounts of antigen were used in each case to test for skin reactivity (that is, 10 to 20 $\mu$g of dextran and 0.8 to 1.6 $\mu$g SVII). Therefore, although the results in the dextran and SVII systems are not strictly interchangeable, they do perhaps provide some quantitative basis for the allergist's definition of a very "sensitive" individual as one who reacts to less antigen than the less "sensitive" individual.

In an effort to determine whether route of immunization played a role in the development of respiratory allergic disease in man, one of the antigens (dextran) was given intradermally. This procedure, short of the more difficult intranasal inoculation or instillation, would be most likely to provide a lymphatic stimulation similar to that following mucosal absorption.[9] The results indicate no special virtue for this method of immunization, and indeed they are similar both as to amounts of antibody and skin reactivity to those produced by Kabat and Berg[4] and Maurer[4a] using subcutaneous inoculation. It must, however, be realized that phenomena other than lymphatic spread may be involved in the differences between intradermal and transmucosal immunization. In addition, although the polysaccharides used here have the virtue of being pure, safe, and easily measured, there may exist differences in the way such antigens are handled in man as compared to proteins. Thus the similarities found in this study might only obtain for polysaccharide antigens given by injection. It would be of considerable interest to attempt a similar study using nasal instillation as a mode of immunization.

While no important differences in immunologic response between the groups was found in this study, the rough correlation between antibody levels and skin reactivity is noteworthy. (Recent studies[10, 11] have indicated a similar parallel between the titer of antibody for pollen extract by the red cell agglutination technique and the degree of allergy.)

The question of the identity or nonidentity of the precipitating antibody and the skin-sensitizing antibody in the present system is crucial and will require further investigation. Since recent studies[10, 11] have indicated a similar parallel between titer of circulating antibody and clinical allergic disease, the answer to this question might help decide whether allergy is dependent only on production of enough of the appropriate antibody, or whether only "allergic people" can develop these antibodies.

SUMMARY

A comparison of the immunologic response of allergic and normal individuals to dextran and SVII polysaccharide revealed no differences between the groups in respect to precipitin antibody or skin reactivity produced to these antigens.

Both groups gave similar skin reactions when tested with histamine, serotonin, and Compound 48/80.

REFERENCES

1. Heidelberger, M., MacLeod, C. M., Hodges, R. G., Bernhard, W. G., and Di Lapi, M. M.: Antibody Formation in Man Following Injection of Four Type Specific Polysaccharides of Pneumococcus, J. Exper. Med. **85:** 227, 1947.
2. Larson, D. L., and Tomlinson, L. J.: Quantitative Antibody Studies in Man, J. Clin. Invest. **32:** 317, 1953.
3. Leskowitz, S., Phillipino, L., Hendrick, G., and Graham, J. B.: Immune Response in Patients With Cancer, Cancer **10:** 1103, 1957.
4. Kabat, E. A., and Berg, D.: Dextran—An Antigen in Man, J. Immunol. **70:** 514, 1953.
4a. Maurer, P. H.: Dextran—An Antigen in Man, Proc. Soc. Exper. Biol. & Med. **83:** 879, 1953.
5. Rapaport, H. G.: Heredity and Allergy. *in* Fundamentals of Modern Allergy, edited by S. J. Priegal, New York, 1960, McGraw-Hill Book Co., Inc., chapt. 7.
6. Kabat, E. A.: Problems in the Study of Allergic Antibodies. Proceedings of the 3rd International Congress of Allergology, edited by B. N. Halpern and A. Holtzer, Paris, 1958, Éditions Médicales Flammarion, pp. 167-177.
7. Farmer, L. M. D.: Evaluation of the Histamine Intradermal Test as a General Indicator of Allergy, J. ALLERGY **16:** 44, 1945.
8. Lecomte, J.: Liberation of Endogenous Histamine in Man, J. ALLERGY **28:** 102, 1957.
9. Yoffey, J. M., and Courtice, F. C.: Lymphatics, Lymph, and Lymphoid Tissues, Cambridge, 1956, Harvard University Press.
10. Gordon, J., Rose, B., and Schon, A. H.: Detection of Non-precipitating Antibodies in Sera of Individuals Allergic to Ragweed Pollen by an In Vitro Method, J. Exper. Med. **108:** 37, 1958.
11. Arbesman, C. E., Rose, N. R., Kantor, S. Z., and Beede, R. B.: Immunologic Studies of Ragweed-Sensitive Patients, J. ALLERGY **31:** 317, 1960.

# THE EXPIRATORY RATE DURING THE THIRD QUARTER OF A MAXIMAL FORCED EXPIRATION ($E_{50-75}$)

## A Useful Index of Obstructive Pulmonary Disease

*William Franklin, M.D., and Francis C. Lowell, M.D., Boston, Mass.*

OBSTRUCTIVE pulmonary disease is one of the most important causes of dyspnea, and is a common reason for patients to seek medical attention. Since mild obstructive disease often goes unrecognized, there is need for a means of detecting its presence and grading its severity. When severe, it is often confused with congestive heart failure, and the common coexistence of the two imposes the urgent need for a ready means of assessing the pulmonary component of the patient's disease.

Although obstructive pulmonary disease produces a great many characteristic aberrations in function, from the clinical standpoint, the most practicable tests are those which measure the maximal expiratory rate. A kymographic tracing of a maximal forced expiration (expirogram)[1] serves this purpose well and we believe that, among the procedures suitable for day to day use in the practice of medicine, this is the best available measure of airway obstruction.

Expirograms in severe emphysema or asthma present an abnormality so gross as to be recognizable at a glance. However, there are many instances which differ from the normal in minor respects and their evaluation requires carefully selected standards to distinguish normal variation from disease. Several parameters, all derivable from the expirogram, have been used as measures of the abnormality in obstructive disease. Some of these are shown in Fig. 1. The volume expired in the first second (FSV)[2] and the rate of expiration between the points where 200 c.c. and 1,100 c.c. have been expired[3] have three disadvantages. First, they deal with the early phase of the expiratory effort at which time the bronchial lumen is most patent and the effect of an obstructing lesion is minimal. Second, patients often fail to exhale as rapidly as possible during early expiration either because of poor motivation or technique. Finally, although of doubtful clinical importance, the inertia of most spirometers makes this portion of the curve differ somewhat from the true maximal expiratory flow rate. Examination of many expiratory tracings from patients with obstructive disease and apparently

From the Departments of Medicine, Harvard Medical School, and Massachusetts General Hospital, Immunology Unit, Boston, Mass.

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normal individuals has shown that errors in performance occur most often in early expiration. This often leads to misleading measurements as shown in Fig. 1, *B*, and such errors will usually go unrecognized unless a tracing is made. A more useful value can be obtained in such instances by extrapolating the steepest portion of the tracing to the base line. Leuallen and Fowler[4] have advocated the use of the maximum expiratory flow rate as a means of avoiding some of these difficulties. This is the average rate of expiration between the points where 25 per cent and 75 per cent of the vital capacity have been expired ($E_{25-75}$), illustrated in Fig. 1, *C*. Because of these considerations and because consistent performance is more readily attained as expiration proceeds, we have studied the average expiratory flow rate between the

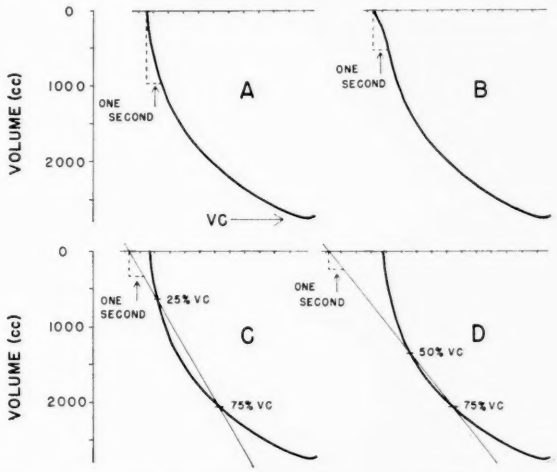


Fig. 1.—Method of obtaining measurements from a kymographic tracing of a maximal forced expiration. The paper moves from right to left at a rate such that the intervals on the base line are equivalent to one second. Expiration is downward.

*A*, Expiratory tracing obtained from a subject with severe obstructive pulmonary disease. The vital capacity (VC) is read from the scale on the left and in this instance is 2,800 c.c. The first second volume (FSV) is obtained by dropping a perpendicular from the point on the base line at which expiration starts and finding a second point determined by deviation of the tracing from the perpendicular by a distance (1 cm.) equivalent to one second. The FSV is then read from the scale at the left and in this instance is 950 c.c.

*B*, The tracing shown in *A* has been purposely altered to illustrate the error introduced if the expiratory rate is not maximal from the start. This is a common error in the performance of these tests. In this instance the FSV is only 550 c.c. It may be seen by inspection that extrapolation of the steepest portion of the curve to the base line will give a tracing which closely resembles that in *A* and a value for the FSV which is less in error.

*C*, $E_{25-75}$ is obtained by drawing a line through the points at which 25 per cent and 75 per cent of the VC have been expired and extending it to the base line. A perpendicular is dropped from the point of intersection. A point is found on the line drawn through the two points such that its distance from the perpendicular is equivalent to 1 second. The $E_{25-75}$ expressed in c.c./sec. is obtained from the scale to the left and in this instance is 300 c.c./sec.

*D*, $E_{50-75}$ is derived in a similar manner after drawing a line through the points at which 50 per cent and 75 per cent of the VC have been expired and in this instance is 250 c.c./sec.

points where 50 per cent and 75 per cent of the vital capacity have been expired ($E_{50-75}$), hoping that this would give a more meaningful single numerical index of obstruction and would be helpful in detecting mild or latent disease (Fig. 1, *D*). Hyatt and co-workers[5] showed that this phase of forced expiration is difficult to distort even when an obstruction is deliberately placed between the mouth and spirometer.

It is the purpose of this paper to compare values for the vital capacity (VC), the first second volume (FSV), "the maximum mid-expiratory flow"

($E_{25-75}$), and the maximum average rate during the third quarter of expiration ($E_{50-75}$) obtained in patients with asthma and emphysema and in apparently healthy subjects.

## MATERIALS AND METHODS

A. *Selection of Subjects.*—One form of obstructive pulmonary disease under consideration occurs predominantly in males over the age of 40. Therefore, all subjects selected were men between the ages of 40 and 60.

1. *Normal subjects:* As part of a study of respiratory and allergic disease,[6] expirograms were obtained in employees of a large industrial plant in Boston. This was done in the course of a routine annual or pre-employment medical examination, which included x-ray examination of the chest. Those with clinical evidence of pulmonary or cardiac disease of a type which might influence pulmonary function were eliminated from consideration without reference to their actual performance.

2. *Patients with obstructive disease* were selected from our practices, merely for the purpose of comparison and not in the belief that the entire range of abnormality occurring in these diseases was to be found among them or that the mean values of the parameters measured typified the disease. All patients had dyspnea, and most had cough and wheezing. There were 9 patients considered to have asthma and thirteen patients, emphysema. They were classified as having asthma if there were associated allergic manifestations, such as allergic rhinitis, nasal polyposis, eosinophilia, and clear evidence by history and skin tests that symptoms were caused or aggravated by environmental allergens. Patients with a high degree of reversibility and peripheral eosinophilia but with no other evidence of atopy were excluded because they could not be classified with certainty.

Patients were considered to have emphysema if they developed primarily exertional dyspnea after the age of 40 and lacked evidence for an allergic component. All patients in this group were "heavy smokers" of cigarettes, although this was not used as a criterion in their selection. Of the patients with asthma, only three were smokers of cigarettes and the remainder did not smoke.

B. *Method of Obtaining Expiratory Tracings.*—Tracings of forced expiration following a maximal inspiration were obtained using a recording spirometer with a kymograph moving at 1 cm. per second (Collins Vitalometer). Usually, two tracings were made but, in some instances, more were required before reproducible curves were obtained. Five or six inhalations of a bronchodilator aerosol were then administered and, after an interval of one to two minutes, two or more tracings were again obtained.

C. *Measurements.*—Measurements were made of the four parameters mentioned above and illustrated in Fig. 1. Among the two or more tracings obtained before the administration of a bronchodilator by aerosol, the one giving the highest value for each of these parameters was selected, irrespective of the value it

gave for the others. Thus the value for the FSV might be obtained from one tracing and that for the VC from another. The first second volume was "corrected" by measuring from the point at which a line, extending the steepest portion of the tracing, intersected the base line.

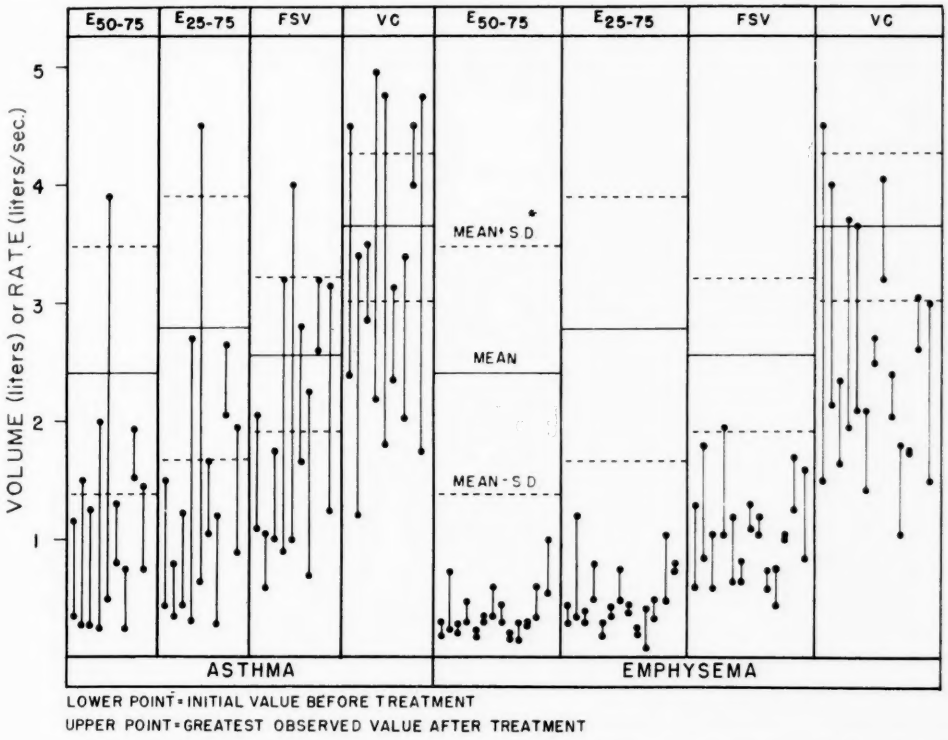


Fig. 2.—Change following treatment in parameters obtained from kymographic tracings of forced expiration in patients with obstructive pulmonary disease. *Solid horizontal line* represents the mean value for the parameter obtained from 213 healthy males, aged 40 to 60, employed at an industrial plant in Boston. *Dashed lines* are drawn one standard deviation above and below the mean.

## RESULTS AND DISCUSSION

In Fig. 2 are shown the results obtained in nine patients with bronchial asthma and thirteen with emphysema. The point at the lower end of each vertical bar represents the value for the measurement in question obtained before the institution of treatment, and before the administration of a bronchodilator aerosol. The upper end of each vertical line represents the best value obtained after prolonged treatment and immediately after the administration of an aerosol of isopropylarterenol. The horizontal lines in the upper portion of each panel represent the mean value obtained from 213 normal males, age 40 to 60, together with lines drawn one standard deviation above and below the mean.[6]

As shown in Fig. 2, the groups with asthma and emphysema gave evidence of similar derangements in each of the parameters, although the initial abnormality was greater in the group with emphysema. More striking, however, was the difference in reversibility of the two diseases indicated

by the length of the vertical lines. Our criteria for the differentiation of asthma from emphysema apparently enabled us to separate patients into two groups which were strikingly different from the standpoint of reversibility of the pulmonary dysfunction measured in this manner. This is clearly shown in Fig. 2 and was most striking for $E_{50-75}$. However, these cases were collected retrospectively and the belief that a high degree of reversibility is characteristic of asthma and not emphysema may have influenced the classification of the cases. Likewise, the search for a history of another manifestation of atopy may have been more avid in the patient whose pulmonary function returned to the normal range after intensive treatment. In order to determine whether there are two forms of bronchial obstruction, one atopic and associated with a high degree of reversibility and the other nonatopic and irreversible, the presence or absence of atopy would have to have been determined without knowledge of the degree of reversibility. The coincidence of atopy with emphysema, both common disorders, will make many cases difficult to classify. In such instances the duration of dyspnea may be helpful. Among the asthmatic patients included in this study, the relative reversibility of bronchial obstruction was especially noteworthy because the average duration of manifest disease was greater (average, twenty years) than in those with emphysema (average, six years). However, it is also true that severe irreversible obstruction may result from long-standing asthma.

Although this means of presentation emphasizes the relative irreversibility of emphysema, the improvement that can be achieved with treatment, though small, is often of great value to the patient. From the standpoint of disability alone, the $E_{50-75}$ may not be so meaningful a measure as the FSV because patients with marked bronchial obstruction partially compensate for their difficulty by shifting their respiratory excursions toward the position of inspiration, which has the effect of facilitating airflow.

TABLE I. MEAN VALUES FOR PARAMETERS OBTAINED FROM THE EXPIROGRAM IN NORMAL SUBJECTS* AND IN PATIENTS† WITH ASTHMA AND EMPHYSEMA

| PARAMETER | OBSERVED VALUES | | | RATIO | |
|---|---|---|---|---|---|
| | NORMAL (213) | EMPHYSEMA (13) | ASTHMA (9) | NORMAL/ EMPHYSEMA | NORMAL/ ASTHMA |
| $E_{50-75}$ (c.c./sec.) | 2,418 | 278 | 550 | 8.70 | 4.40 |
| $E_{25-75}$ (c.c./sec.) | 2,780 | 376 | 734 | 7.39 | 3.78 |
| FSV (c.c.) | 2,565 | 826 | 1,216 | 3.11 | 2.11 |
| VC (c.c.) | 3,646 | 1,958 | 2,246 | 1.86 | 1.62 |

*All subjects were males over 40 years of age.
†Values obtained from Fig. 2 before treatment was initiated.

The mean initial value for each of the parameters and the ratio of the average normal value to this are shown in Table I. The larger the ratio the more sensitive is the parameter as a means of detecting the disease. The $E_{50-75}$ is the most sensitive of the four parameters, and although the difference between it and $E_{25-75}$ may not be important, it is over twice as sensitive as the FSV.

However, a measurement may be highly sensitive but so variable from test to test that it is an unreliable index of a slight deviation from normal or

of a small change in the patient's condition. In Table II are listed the mean differences between successive trials for tests done in patients with emphysema, patients with asthma, and in normal individuals serving as controls. Several pairs of tracings were studied in most patients with asthma and emphysema. To minimize variations due to technique, control subjects selected from hospital personnel were tested under the same circumstances as the patients. In the normals, both $E_{50-75}$ and $E_{25-75}$ were less reproducible than the corrected FSV and VC. This is probably attributable to difficulty in measuring steep slopes accurately. If a kymographic speed much greater than 1 cm. per second were used, this error could be reduced, but then tracings from patients with severe obstructive diseases would be very extended and they would become cumbersome to handle. In the patients with asthma and emphysema, $E_{50-75}$ was the most reproducible of the parameters measured, perhaps largely due to greater accuracy in measurement of these parameters at slower rates of expiration. At this level, therefore, these parameters appear to be not only more indicators of obstructive disease but more reproducible as well.

TABLE II. REPRODUCIBILITY OF PARAMETERS OBTAINABLE FROM THE EXPIROGRAM IN SELECTED CONTROLS AND PATIENTS

| | AVERAGE DIFFERENCE BETWEEN TWO SUCCESSIVE TRIALS | | | | | |
|---|---|---|---|---|---|---|
| | WITHOUT REGARD TO SIGN | | | WITH REGARD TO SIGN | | |
| | CONTROLS | EMPHYSEMA | ASTHMA | CONTROLS | EMPHYSEMA | ASTHMA |
| | 12* | 13 | 9 | 12 | 13 | 9 |
| PARAMETER | 12 | 40 | 35 | 12 | 40 | 35 |
| $E_{50-75}$ (c.c./sec.) | 306 | 46 | 117 | −150 | −13 | −7 |
| $E_{25-75}$ (c.c./sec.) | 306 | 74 | 138 | −174 | −14 | −37 |
| FSV (c.c.) | 102 | 107 | 128 | +37 | 0 | −4 |
| VC (c.c.) | 107 | 164 | 170 | +51 | +60 | −37 |

*Upper figure is number of individuals: lower is number of trials.

In the second part of Table II the mean difference between the second trial and first trial is given with regard to sign. A significantly positive difference may represent the effect of training and, conversely, a significantly negative value, the effect of fatigue or increased bronchial obstruction induced by the first trial. In patients with asthma and emphysema it would seem that of the parameters studied, the $E_{50-75}$ changed least, although the $E_{25-75}$ also changed little whereas the FSV gave considerable variation. It appears, therefore, that the $E_{50-75}$ deserves consideration as a means of following changes in the status of such patients.

Although, as already mentioned, $E_{50-75}$ and $E_{25-75}$ are less reproducible in normals than is the VC or FSV, the greater sensitivity of the first two parameters probably more than compensates for their lesser reproducibility. Furthermore, the relatively high degree of reproducibility indicated by the values in Table II for the FSV is somewhat spurious because the measurements were corrected by extrapolating the steepest portion of the tracing to the base line.

Some interval other than the points where 50 per cent and 75 per cent of the vital capacity has been expired, perhaps closer to the point of end-expiration, may be more suitable. Greater sensitivity could perhaps be

achieved but this might also be at the expense of greater variability. A large interval is required in order that errors in measurement do not become too great and we are doubtful that selection of other points will give a significantly more useful index.

We believe, therefore, that $E_{50-75}$ is the preferred parameter for detecting lesser degrees of bronchial obstruction. It would appear to be especially useful in detecting minimal or latent asthma or emphysema in evaluating the role of factors in the environment that have been held responsible for inducing bronchial obstruction, such as smoking or air pollution. The relationship of smoking to changes in $E_{50-75}$ has been studied[6] and the relationship of this parameter to age, sex, and height in a factory population is under investigation.

## SUMMARY AND CONCLUSIONS

The expiratory flow rate during the third quarter of a maximal forced expiration ($E_{50-75}$) was studied in normal individuals and in patients with asthma and emphysema. Its sensitivity and reproducibility were compared with that of other commonly used parameters, and evidence that $E_{50-75}$ is a more useful index of bronchial obstruction is presented. Furthermore, the change in this parameter after intensive treatment of the patient may be an important means of differentiating asthma from chronic obstructive emphysema.

We, therefore, believe that this measurement is helpful in the diagnosis of asthma and emphysema, in estimating the severity of these diseases, and in following changes in the patient's status.

## REFERENCES

1. Franklin, W., Michelson, A. L., and Lowell, F. C.: Clinical Value of a Tracing of Forced Expiration (Expirogram). I. Pulmonary Disease. New England J. Med. **253:** 799-808, 1955.
2. Gaensler, E. A.: Analysis of Ventilatory Defect by Timed Capacity Measurements, Am. Rev. Tuberc. **64:** 256-278, 1951.
3. Comroe, J. H., Jr., et al.: The Lung: Clinical Physiology and Pulmonary Function Tests, Chicago, 1955, Year Book Publishers.
4. Leuallen, E. C., and Fowler, W. S.: Maximal Midexpiratory Flow, Am. Rev. Tuberc. **72:** 783-800, 1955.
5. Hyatt, R. E., Schilder, D. P., and Fry, D. L.: Relationship Between Maximum Expiratory Flow and Degree of Lung Inflation, J. Appl. Physiol. **13:** 331-336, 1958.
6. Franklin, W., and Lowell, F. C.: Unrecognized Airway Obstruction Associated With Smoking: A Probable Forerunner of Obstructive Pulmonary Emphysema, Ann. Int. Med. (In press.)

# CORRESPONDENCE

## A Plea for More Scientific Evaluation of Problems in Clinical Allergy

New York University Medical Center
New York 22, N. Y.

To the Editor:

It should be of concern to the physician practicing allergy that his associates in internal medicine, pediatrics, and general practice have looked upon the allergist with considerable suspicion and lack of understanding in the past many years. I believe that much of this suspicion has stemmed from the many reports in the allergy literature which have presented conclusions which have been inadequately supported. If the allergist is to be better recognized by his colleagues in medicine, problems in the field of allergy must be clearly visualized, impartially studied, and more scientifically evaluated. Only in this way can the physician in the practice of allergy earn the respect of his colleagues.

The proponents of repository therapy for hay fever claim this to be as effective or more effective than the procedure entailing multiple injections of aqueous extracts. Although this last has been practiced for a generation, there are wide differences of opinion among the medical profession as a whole regarding its effectiveness. To be sure, allergists, in general, regard this type of therapy highly. On the other hand, specialists in the field of medicine, many pediatricians, and others entertain considerable skepticism as to its value and point out that the improvement which follows therapy is neither striking nor constant, that many individuals who are not given the injections show symptomatic improvement quite comparable to those so treated, and that suggestion may play an important role. For the assessment of efficacy in so complex a situation, a "double blind" type of study is probably essential.

To the best of my knowledge, no acceptable double-blind, controlled study of conventional treatment for hay fever has been made. How, therefore, can the proponents of repository therapy compare their results with conventional treatment in the absence of suitably controlled studies indicating the latter's effectiveness. The logical approach would be first to evaluate conventional therapy by an impartial study and, if the results warrant it, then to assess repository treatment.

The clinical investigator can hardly avoid bias, which may lead him to either overestimate or underestimate the significance of his findings. The medical statistician may be of great help to the clinical investigator in determining whether the response of the patient is a result of treatment, of chance, or of other factors. The application of proper experimental design and statistical analysis will guard against invalid inferences being drawn.

It is in the early phases of planning a study that the contribution of the statistician is especially important. When properly set up and organized, a study is far more likely to yield meaningful results and such an outcome is promoted when there is complete understanding between the various members concerned as to aims, methods of procedure, types of patients, duration of trial, the means of measuring effects, and the choice of materials to be used. The conduct of the investigation must be clearly outlined, including the role to be played by the personnel responsible for the various aspects of the study.

The future of allergy depends on careful, honest, and scientific evaluation of clinical problems in allergy by the interested investigator. The approach outlined above, far from discouraging research efforts in the field of allergy, should stimulate a more intensified effort on the part of the clinical investigator.

The challenge facing the allergist in clinical research today can best be described by the statement of an eminent historian of science, Benjamin Farrington, who wrote:

> In the infinite variety and complexity of the phenomena of nature, the scientist is at a loss in which direction to turn unless he is looking for something. If he is looking for something, that means he has a theory. If he has a theory, he tends to see what supports it and to miss other significant facts. There is no way out of this difficulty except the patience and discipline which the long tradition of science can help to supply. In this situation an ardent and enthusiastic mind is more likely to error than one that is without these attractive qualities.

The unfortunate publicity that has surrounded the emulsion type of therapy has done damage to the stature and integrity of the subspecialty of allergy. I believe that the challenge that now faces the American Academy of Allergy and other allergy groups throughout the country is to prove to the medical profession, by careful study, that injection therapy is effective, useful, and without danger.

Nov. 28, 1960

VINCENT J. FONTANA, M.D.
150 East 56th St.
New York 22, N. Y.

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60 East 58th St.
New York 22, N. Y.

TO THE EDITOR:

In the article, "Immune Milk in the Treatment of Hay Fever," by Dr. Samuel Freedman and myself, published in the September 1960 issue of THE JOURNAL OF ALLERGY, we mentioned The Borden Company as supplying materials and assistance in the investigation reported.

Since the article also states that protective milk has been exploited commercially for the treatment of hay fever, I would like to make it clear that The Borden Company has had no part in this commercial exploitation. The Borden Company was interested and helpful in the scientific appraisal of this product but has had no further interest since clinical trials proved it to be ineffective.

I hope that this letter can be pubished in THE JOURNAL in justice to The Borden Company.

Dec. 14, 1960

WILLIAM B. SHERMAN, M.D.

## OBITUARIES

### LOUIS BEARDSLEE BALDWIN

Louis Beardsley Baldwin, Senior, died on Oct. 24, 1960, in Phoenix, Arizona, where he had practiced for many years. Memorial services were held for him in Trinity Episcopal Cathedral in Phoenix.

Dr. Baldwin was born on Dec. 3, 1890, in Florence, Italy, where his father was a practicing physician, and came to the United States at the age of 11 years. He attended Princeton University from which he received the degree of Litt. B. and secured his M.D. degree at the College of Physicians and Surgeons, Columbia University, in 1919. He interned at the Presbyterian Hospital, New York City, followed by a residency at the Peter Bent Brigham Hospital, Boston.

After completion of his residency in 1923, he took up the practice of Internal Medicine in Rochester, New York. Between 1923 and 1930 Dr. Baldwin taught in the School of Medicine and Dentistry, University of Rochester, where he came to hold the rank of Assistant Professor of Medicine. He was on the staff of the Rochester General Hospital and of the Strong Memorial Hospital and he collaborated with Dr. Stearns S. Bullen in the founding of the Allergy Clinic at Strong Memorial.

In 1934 he removed to Arizona to become Chief of Medicine at the Desert Sanitarium in Tucson, remaining there until 1937. Thereafter he lived and practiced in Phoenix, where he was on the staffs of the Good Samaritan and St. Joseph's Hospitals. He was certified in Internal Medicine in 1937.

He was a Fellow of the American Academy of Allergy, having joined in 1924, the Society for the Study of Asthma and Allied Conditions, one of the two parent societies of the Academy.

In Phoenix, he was a founder of the Salt River Valley Blood Bank. He was president of the Maricopa County Medical Society from 1941 to 1943, and was president-emeritus of the Pacific Interurban Clinical Club of San Francisco.

Besides membership in his county and state medical societies, he was a Fellow of the American College of Physicians, and a member of the American Diabetic Association, the American Heart Association, The Southern Medical Association, and the American Medical Association.

Surviving Dr. Baldwin are a daughter, two sons, and seven grandchildren, as well as two sisters and two brothers.

*H. O.*

## LINN FENIMORE COOPER

Linn Fenimore Cooper of Washington, D. C., died on Nov. 13, 1959, in Sarawak, on the northwest coast of Borneo. He had been ill of encephalitis for only a few days.

Dr. Cooper, a great-grandson of the American novelist, James Fenimore Cooper, was born in Albany, New York, on May 5, 1899, to James Fenimore Cooper and Susan Sage Cooper. He graduated from Yale University with a Bachelor of Arts degree in 1921 and received his medical degree from Harvard University in 1925. He interned at the Massachusetts General Hospital 1926-1928, and was assistant resident in medicine at Johns Hopkins Hospital, 1928-1930.

An internist, he practiced in Washington, D. C., where he was on the medical staffs of Georgetown University Hospital and the old Washington Emergency Hospital. He was Medical Director of the Washington Home for Incurables from 1938-1941. He was Associate Professor of Clinical Medicine at Georgetown University for many years.

During World War II, Dr. Cooper entered service as Major in the Medical Corps of the Army in 1942 and was separated as Colonel four years later. After his discharge, he went to China as Professor of Medicine at Yale-in-China in Chang-sha, where he remained until shortly after the Communists seized power. For a time he again taught medicine at Georgetown University, where he also carried on some experimental research in hypnosis. Later he served with Catholic medical missions in Ghana, Africa, and in Borneo. Although not himself a Catholic, he gave his services to Catholic missions because he wanted to use his medical skills where they were needed, and did not feel competent to act both as clergyman and physician, as is common in most Protestant missions.

Although not an allergist, he appreciated the fundamental importance of allergy in Internal Medicine and impressed this on his students. He became a member of the Academy in 1952. He was an active member of the Medical Society of the District of Columbia and a member of the American Medical Association.

Surviving him are two brothers, Dr. Henry S. Fenimore Cooper of New York City and Dr. Paul Fenimore Cooper of Cooperstown, New York.

*H. O.*

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## LEONARD H. HARRIS

Leonard H. Harris of Peoria, Ill., died on Dec. 4, 1960. Dr. Harris was born in Ada, Oklahoma, in 1905. He graduated from Northwestern University Medical School in 1933. After an internship at Cook County Hospital (Chicago) followed by a residency in pediatrics at the same institution, he began the practice of pediatrics in Elyria, Ohio. Impressed with the importance of allergy

in pediatries he took training with Dr. Milton Cole, then practicing in Cleveland. Thereafter Dr. Harris confined himself to the practice of allergy. In 1938, he held the office of Secretary, and, in 1939, President of the Cleveland Allergy Society. From 1942 to 1945 Dr. Harris served in World War II as lieutenant commander in the Navy Medical Corps and spent most of that time at Pearl Harbor in charge of allergy in the Hawaiian Theatre. Upon his return from service he settled in Peoria where he practiced until the day of his death.

From 1945 to 1951 Dr. Harris was a faculty member of Northwestern University Medical School, participating in the teaching activities of the Allergy Clinic, and he made weekly trips to Chicago, even though the round trip is nearly 400 miles. He was a member of local and national societies and a Fellow of the American Academy of Allergy since 1940. His interests in allergy were many and he took special early interest in clinical experiments in mold allergy and in the grain dust allergy problem. His publications were always careful, conservative, and modest in claims.

As one who knew him intimately, the writer can attest to the exceptionally fine qualities of the man: a logical thinker, yet gentle in his arguments; possessing a high sense of loyalty to his friends, respect for his colleagues, and totally devoid of animosity or harsh words. All who knew Len Harris admired and loved him and his image and spirit will long endure with them.

He is survived by his widow, Cecile, his daughters, Louise Trum and Barbara Gordon, and four grandchildren.

*S. M. F.*

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## JOHN HENRY TRESCHER

John Henry Trescher, Fellow of the American Academy of Allergy, died at the age of 60 years on Nov. 15, 1959, in Baltimore, Maryland. Dr. Trescher was born in Jiannette, Pennsylvania. After attending local and private schools, he was graduated from Cornell University, Ithaca, New York, in 1921, and from the Cornell University Medical School in 1924. Following an internship at the Allegheny General Hospital, Dr. Trescher served as an intern on the private medical service at The Johns Hopkins Hospital. Here he frequently came in contact with one of his teachers, the late Dr. Llewellyn F. Barker, who persuaded Dr. Trescher to enter the private practice of medicine as an associate in his office.

Dr. Trescher held a large practice and, in addition, was very active as a member of the staff of The Johns Hopkins Hospital. Dr. Trescher was appointed Assistant Dispensary Physician in 1928 and later Dispensary Physician, and concurrently held the rank of instructor in The Johns Hopkins Medical School. He was an enthusiastic participant in the Dispensary Clinics for over 30 years, where he became especially interested in diabetes and allergic diseases. In 1938, he received his appointment as Visiting Physician to The Johns Hopkins Hospital. Dr. Trescher was also a member of the staffs of several other

hospitals in Baltimore: The Union Memorial Hospital, The Sinai Hospital, The Hospital for the Women of Maryland, and The Church Home and Hospital. Dr. Trescher was a member of The American Medical Association and, later, the Diabetes Association. He became a member of the Society for the Study of Asthma and Allied Conditions in 1939 and, in 1952, he was advanced to Fellowship in The American Academy of Allergy. For social activities, he was long a member of The Elkridge Club of Baltimore, and The Johns Hopkins Faculty Club.

Although Dr. Trescher possessed wide scientific interests and led a busy professional life, he was first of all a clinician and, parenthetically, a good one, and was respected and loved alike by his associates and his many patients. His medical papers covered subjects in adrenal physiology, performed in association with the late Dr. George Harrop of The Johns Hopkins Medical School, problems in food allergy, and certain aspects of diabetes. Dr. Trescher was also co-author with the late Dr. Llewellyn F. Barker of the volume on *Backache* and he further contributed to *Every Day Practice Series* (J. B. Lippincott Company, 1931), edited then by Dr. Harlow Brooks. During World War II, Dr. Trescher served as Chairman of The Johns Hopkins Medical Advisory Board of The Selective Service System and gave untiringly of his energies to this work during the war period.

During the later years of his active life, Dr. Trescher was handicapped by progressive deafness; this, however, did not detract from his continued interests and activities in hospital and office work until he was eventually forced—and reluctantly so—to stop when his final illness showed itself. Dr. Trescher's friends and all who knew him so well miss his cheerful presence, his unfailing good humor, and mourn his untimely death.

Dr. Trescher is survived by his widow, Mrs. Eloise Rodney Trescher, of Baltimore, Maryland, and his daughter, Mrs. John A. Waldhausen of Indianapolis, Indiana.

*W. L. W.*

# ANNOUNCEMENTS

## Officers of The New York Allergy Society, 1960-1961

At its Annual Meeting, Nov. 16, 1960, The New York Allergy Society elected the following officers.

*President:* Dr. Joseph H. Fries
*President-Elect:* Dr. Sheppard Siegal
*Vice-President:* Dr. Murray Dworetzky
*Secretary:* Dr. Ely Perlman
*Treasurer:* Dr. Walter Kessler
*Assistant Secretary-Treasurer:* Dr. Bernard Siegel

The address of the secretary is 118 Crescent Lane, Roslyn Heights, N. Y.

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## IVth International Congress of Allergology

The IVth International Congress of Allergology will be held at the Hotel Commodore, New York City, Oct. 15-20, 1961. It is anticipated that this will be a large and interesting meeting for all of those concerned with allergic diseases and related fields of immunology. At the main meetings, there will be simultaneous interpretation of all papers in English, French, German, and Spanish. Prominent physicians and scientists, from all parts of the world, have been invited to take part in conferences, symposia, and panel discussions. Among the subjects to be presented are:

> Genetics in Allergy; Acquired Tolerance; Transplantation Immunity; Drug Hypersensitivity; Contact Allergy; General Mechanisms in Allergy; Mechanisms of Antibody Fixation; Delayed Hypersensitivity; Autoimmune Processes; Steroid Therapy; New Methods in Allergy, among others.

All physicians registering are invited to present communications which will be grouped in various sections according to subject matter. An active program of entertainment is being arranged with several receptions, one at the Metropolitan Museum of Art, and a Banquet. For the ladies, there will be a program of luncheons, fashion shows, and visits to the United Nations and other points of interest.

The registration fee for regular members will be $45.00, for wives $20.00. These registration fees will include the printed proceedings and admission to the receptions. The banquet will be charged separately. As the attendance is expected to be large, it is requested that persons interested obtain additional information from Dr. William B. Sherman, 60 East 58th Street, New York 22, New York.

## American Medical Association Session in Allergy

All those desiring to submit abstracts for the session in allergy of the American Medical Association in New York City in June, 1961, should forward a 250 word abstract in triplicate to Murray Dworetzky, M.D., Secretary, 115 East 61st Street, New York 21, New York.